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Egbert Wilson Hollingsworth
Iowa State College

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PREPARATION OF VARIOUS CHOLESTEROLS, CHOLESTADIFERUS
AND CHOLESTADRINES

by

Reginald Wilson Hollingsworth

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject Agricultural Chemistry

Approved:

Signature was redacted for privacy.

In charge of Major work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College

1931
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. INTRODUCTION</td>
<td>6</td>
</tr>
<tr>
<td>II. HISTORICAL</td>
<td>8</td>
</tr>
<tr>
<td>A. Unsaturated Cholesterol Derivatives</td>
<td>6</td>
</tr>
<tr>
<td>1. Cholestenes</td>
<td>6</td>
</tr>
<tr>
<td>a. $\Delta^\text{7}$-Cholestenes</td>
<td>8</td>
</tr>
<tr>
<td>b. $\Delta^\text{2}$-Cholestenes</td>
<td>10</td>
</tr>
<tr>
<td>c. $\Delta^\text{5}$-Cholestenes</td>
<td>10</td>
</tr>
<tr>
<td>2. Cholestadienes</td>
<td>11</td>
</tr>
<tr>
<td>a. $\Delta^\text{7}$-Cholestadiene</td>
<td>11</td>
</tr>
<tr>
<td>b. $\Delta^\text{2}$-Cholestadiene</td>
<td>12</td>
</tr>
<tr>
<td>c. $\Delta^\text{5}$-Cholestadiene</td>
<td>13</td>
</tr>
<tr>
<td>d. $\Delta^\text{5}$-Cholestadiene</td>
<td>13</td>
</tr>
<tr>
<td>E. Other Unsaturated Steroid Derivatives</td>
<td>13</td>
</tr>
<tr>
<td>1. Unsaturated Derivatives of Cholestan-3-ol</td>
<td>15</td>
</tr>
<tr>
<td>a. Mono-unsaturated Derivatives of Cholestan-3-ol</td>
<td>15</td>
</tr>
<tr>
<td>b. Di-unsaturated Derivatives of Cholestan-3-ol</td>
<td>16</td>
</tr>
<tr>
<td>2. Unsaturated Derivatives of Coprostan-3-ol</td>
<td>17</td>
</tr>
<tr>
<td>a. Mono-unsaturated Derivatives of Coprostan-3-ol</td>
<td>17</td>
</tr>
<tr>
<td>b. Di-unsaturated Derivatives of Coprostan-3-ol</td>
<td>18</td>
</tr>
<tr>
<td>3. Unsaturated Ergostane Derivatives</td>
<td>19</td>
</tr>
<tr>
<td>a. Mono-unsaturated Ergostane Derivatives</td>
<td>19</td>
</tr>
<tr>
<td>b. Diunsaturated Ergostane Derivatives</td>
<td>20</td>
</tr>
<tr>
<td>c. Isomers of Ergosterol</td>
<td>21</td>
</tr>
<tr>
<td>d. Other Unsaturated Ergostane Derivatives</td>
<td>23</td>
</tr>
</tbody>
</table>
I. Unsaturated Derivatives of Sitostan-3-ol ................. 25
II. Unsaturated Derivatives of Cholanic Acid ................... 26
III. EXPERIMENTAL ............................................ 28

A. Cholestanes ................................................. 28
   1. Preparation of \( \Delta^e \)-Cholestene .................... 28
      a. Preparation of Cholestan-7-ol ....................... 28
      b. Dehydration of Cholestan-7-ol ..................... 29
      c. Conversion of \( \Delta^e \)-Cholestene to Cholestan-7-one .. 31
   2. Preparation of \( \Delta^8(14) \)-Cholestene ............... 33
   3. Preparation of \( \Delta^{14} \)-Cholestene .................. 34
      a. Treatment of \( \Delta^8(14) \)-Cholestene with Hydrogen Chloride .. 34
      b. Treatment of \( \Delta^{14} \)-Cholestene with Hydrogen Chloride .... 35
      c. Catalytic Hydrogenation of \( \Delta^{14} \)-Cholestene .......... 35

4. Bromine and Perbenzoic Acid Titrations of Cholestanes .... 36
   a. Titration with Bromine .................................... 36
   b. Titration with Perbenzoic Acid .......................... 37

B. Cholestadienes ............................................. 37
   1. Characterization of \( \Delta^4,6 \)-Cholestadiene .......... 37
      a. Preparation of \( \Delta^4,6 \)-Cholestadiene ............... 37
      b. Chromic Acid Oxidations .............................. 39
      c. Catalytic Hydrogenation of "7-Dehydrocholestene Isomer" ..... 41
      d. Treatment of "7-Dehydrocholestene Isomer" with
         Hydrogen Chloride ................................... 41
      e. Bromination of the 5,6-Dibromocholestane-quinoline
         Product ............................................... 42
Page

2. Preparation of \( \Delta^{7,9(11)} \)-Cholestadiene ........................................ 43

3. Preparation of \( \Delta^{7,14} \)-Cholestadiene .......................................... 43

4. Preparation of \( \Delta^{6,14} \)-Cholestadiene .......................................... 45
   a. Treatment of \( \Delta^{5(14)} \)-Cholestene with Peroxenic Acid ................. 45
   b. Treatment of \( \Delta^{5(14)} \)-Cholestene with Selenium Dioxide .......... 46
   c. Treatment of \( \Delta^{5(14)} \)-Cholestene with Bromine ................. 46
   d. Treatment of \( \Delta^{5(14)} \)-Cholestene with Chronic Acid ............ 47
   e. Catalytic Hydrogenation of \( \Delta^{8,16} \)-Cholestadiene .............. 47
   f. Treatment of \( \Delta^{8,16} \)-Cholestadiene with Maleic Anhydride ...... 47

5. Bromine Titration of Cholestadienes .............................................. 48

6. Cholestatrienes ................................................................................. 48
   1. Preparation of \( \Delta^{7,9,14} \)-Cholestatriene .................................... 48
   2. Preparation of \( \Delta^{8,15} \)-Cholestatriene ................................... 49
      a. Preparation of \( \Delta^{8} \)-Cholestadiene-7-ol ......................... 49
      b. Dehydration of \( \Delta^{8} \)-Cholestadiene-7-ol ....................... 49

IV. DISCUSSION ...................................................................................... 51

V. SUMMARY .......................................................................................... 57

VI. LITERATURE CITED ........................................................................... 58
I. INTRODUCTION

Relatively few non-substituted unsaturated derivatives of cholestane, the basic hydrocarbon of which cholesterol is a derivative, are known. Thus, only three cholestanes are known although there are fourteen theoretically possible nuclear mono-unsaturated derivatives of cholestane. Likewise, only three cholestadienes are definitely established although there are fourteen theoretically possible conjugated nuclear di-unsaturated derivatives. In addition, no cholestatriene has been characterized.

The known cholestanes possess double bonds located in ring A or B and the known cholestadienes possess double bonds which are located in conjugation in ring A or B or both. In order to render available the desirable cholestane derivatives for investigations of rings C and D, it was desirable to prepare cholestane derivatives which possess unsaturation in rings A and B. Various steroid derivatives unsaturated in rings C and D are known but all of these derivatives, with the exception of only two, possess substitution in the 3-position. This substitution limits the reactions desirable for investigations of rings C and D.

The methods of preparation and the properties, especially specific rotation, of the known steroid derivatives which possess unsaturation in rings C and D are somewhat consistent. However, there are additional derivatives for which the structures have not been suggested. An interpretation of the methods of preparation and properties of the reported steroid derivatives is thus of importance in the preparation and structure proof of cholestane derivatives unsaturated in rings C and D.

It was the purpose of this investigation to prepare and study
various properties of new cholestene, cholestadienes and cholestatrienes. Cholestene derivatives possessing unsaturation in rings C and D were considered to be of most importance.
The methods of preparation and the properties, especially specific rotation, of various steroid derivatives possessing unsaturation in or adjacent to rings C and D were studied. The clarity in presentation of such derivatives is dependent upon the nomenclature as well as upon the orderly arrangement of the discussion. The steroid derivatives involved are dimethyl derivatives of perhydrocyclopenteno-phenanthrene and differ in the side chain, B, at the 17-position as presented in Figure I. The same numbering system is used throughout with all the derivatives discussed. A steroid derivative in which a ring of the nucleus is broken, is designated by a prefix consisting of the numbers of the severed carbon atoms separated by two vertical lines (such as 5|6). The nomenclature and properties of steroids are available in various forms of presentation in a number of reviews(9,31,53,60).

A. Unsaturated Cholestanol Derivatives

1. Cholestanol.

The three cholestanol which have been reported are δ⁴-cholestanol (neo-cholestanol), δ⁶-cholestanol (pseudo-cholestanol or coprostane) and δ⁸-cholestanol (cholestanol).

a. δ⁵-Cholestanol. δ⁵-Cholestanol has been prepared by the action of quinoline on cholesteryl chloride (3-chlorocholestane)(38), by the heat treatment of cholest-3-en (dihydrocholesterol) with acid clay(31) and by refluxing a methanol solution of p-toluenesulfonate(39). δ⁵-Cholestanol adds one mole of bromine to form α-8,9-dibromocholestanol when heated above 175°C(31).
Basic saturated steroid structure of sterols and bile acids.
Debromination of either α- or β,β,5-dibromocholestan to α5-cholestan is accomplished by treatment with zinc and acetic acid(21). Treatment with perbenzoic acid converts α5-cholestan to the 2,3-oxide of cholestan which on hydrolysis with alcoholic hydrochloric acid yields the two isomeric cholesterol-2,3-diones(19). The structure of α5-cholestan was determined by its conversion by chronic acid oxidation to 2[1-2,3-dicarbonylolestan(32) which had been previously obtained by the chronic acid oxidation of dihydrocholesterol (cholestan-3-ol).

b. α5-Cholestan. α5-Cholestan has been prepared by refluxing an alcoholic solution of 5-chlorocholestan (cholestan hydrochloride) with freshly fused sodium acetate(35), by the Wolff-Kishner reduction of the semicarbazone of cholestanes(30) and α4-cholesten-3,6-dione(55), by the sodium-absolute alcohol reduction of 7-chloro-α5-cholestan(76), by the sodium-alcohol reduction of α5-α4-cholestadiene(57) and by the pyrolysis of cholestanol(16,23). α5-Cholestan adds one mole of bromine to form two isomeric 1,5-dibromocholestanones, one of which mutarotates in chloroform solution, apparently to the other isomeric form(35). Chronic acid oxidation of α5-cholestan yields α5-cholesten-3-one, α5-cholesten-7-one(17) and 1[1-5-carboxy-5-heto-cholestan(30). Perbenzoic acid converts α5-cholestan to the 1,5-oxide of cholestan which on catalytic reduction yields cholestan(24) and on treatment with alcoholic hydrochloric acid yields a cholestadiene (referred to as α5-α4-cholestadiene(21). Catalytic hydrogenation of α5-cholestan yields coprostan(21,77).

c. α5-Cholestan. α5-Cholestan has been prepared by the sodium-alkyl alcohol reduction of cholesteryl chloride(37) and by the sodium amalgam-ethyl alcohol reduction of cholesteryl chloride(37). α5-Cholestan adds
one mole of bromine to yield mainly 7- and some 2,5,6-dibromocholestone(36,37). 
7,5,6-dibromocholestone autodecomposes on standing or when warmed in alcohol solution to 5,6-dibromocholestone(36,37). \( \Delta^5 \)-Cholestanone also gives one mole of chlorine to yield 5,6-dichlorocholestone(33) and one mole of hydrogen chloride to yield 5-chlorocholestone(33,35). Chronic acid oxidation of \( \Delta^5 \)-cholestanone gave a 10 per cent yield of \( \Delta^5 \)-cholesten-7-one(33,62,63) and 5|6|6-keto-6-carboxycholestanone(29). Peroxidic acid converts \( \Delta^5 \)-cholestanone in chloroform solution to a mixture of the \( \alpha \)- and \( \alpha \)-5,6-oxides of cholestanone either of which when treated with acetic acid containing sulfuric acid yields 5-carboxycholesten-5-one which on acetylation yields cholestan-5,6-diol(43).

Treatment of the \( \alpha \)-5,6-oxide of cholestanone with alcoholic hydrochloric acid yields a cholestadiene (referred to as \( \Delta^2 \)-cholestadiene)(28). Catalytic hydrogenation of \( \Delta^5 \)-cholestanone and of the \( \alpha \)-5,6-oxide(28) of cholestanone yields cholestanone.

2. Cholestadienes.

The three cholestadienes which have been definitely prepared and characterized are \( \Delta^2 \)-cholestadiene (2,3-coprostanene), \( \Delta^2 \)-cholestadiene and \( \Delta^5 \)-cholestadiene (referred to as 7-dehydrocholestanone). A product referred to as \( \Delta^1 \)-cholestadiene has also been reported but not definitely characterized.

a. \( \Delta^2 \)-Cholestadiene. \( \Delta^2 \)-Cholestadiene has been prepared by the heat treatment of cholesterol with activated alumina(52,57). The structure of \( \Delta^2 \)-cholestadiene was assigned since perbenzic acid titration demonstrated the presence of two double bonds, and the absorption spectra maximum at 260 nm together with the formation of a maleic anhydride addition product demonstrated a conjugated diene system with both double bonds in the same ring(57). \( \Delta^2 \)-Cholestadiene is converted to a stable peroxide by the action
of oxygen in the presence of light and osolin, to A\textsuperscript{3,14}-cholestadiene by the action of alcoholic hydrochloric acid, to A\textsuperscript{1}-cholesten-4-ol by sodium-amyl alcohol reduction and to cholestane by catalytic hydrogenation in ethyl acetate solution with platinum catalyst(57).

b. A\textsuperscript{3,14}-cholestadienes. A\textsuperscript{3,14}-Cholestadiene in a practically pure condition has been prepared by relatively few methods although compounds referred to as A\textsuperscript{3,14}-cholestadiene or as cholesterolene have been prepared by numerous methods. Thus, A\textsuperscript{3,14}-cholestadienes apparently has a specific rotation of -129.6\textdegree and has been prepared in a practically pure condition by the aluminium amalgam reduction of 6-chloro-3-benzoyloxy-A\textsuperscript{4}-cholesten-4-ol in ether(57), by the alcoholic hydrochloric acid dehydration of the molecular compound of all- and epi-all-cholesterol(12) and by the copper nitrate dehydration of cholesterol(12). Compounds referred to as A\textsuperscript{3,14}-cholestadiene which possessed specific rotations from -65.75\textdegree to -105.2\textdegree were prepared by the removal of hydrogen bromide from \textit{l,3}- dibromocholestan by the action of cupric oxide (14) or silver nitrate-pyridine(36) and by the Wolf-Kischner reduction of the semicarbazone of A\textsuperscript{3,14}-cholestadiene-7-one(56). Other products referred to as cholesterolene which possessed specific rotations from -11.45\textdegree to -100.3\textdegree were prepared by numerous methods. The structure of A\textsuperscript{3,14}-cholestadiene was assigned since perbenzoic acid titration demonstrated the presence of two double bonds, and the absorption spectra maxima at 235 m\textmu together with the non-formation of a normal melilite succinate addition product demonstrated a conjugated diene system with two double bonds in two adjoining rings(51,56). A\textsuperscript{3,14}-cholestadiene is unaltered by treatment with sodium and alcohol(63), adds one mole of bromine although no bromine derivative has been isolated(63) and yields A\textsuperscript{1}-cholesten-3,6-dione upon chromic acid
3. \( \Delta^{7} \)-Cholestadiene. \( \Delta^{7} \)-Cholestadiene has been prepared by the pyrolytic cleavage of benzoic acid from \( \Delta^{5} \)-cholester-7-en benzoate(10). Under a less evacuated condition or a higher temperature, the pyrolysis yields "7-hydrocholestanone isomer"(10) which is also obtained by the action of acetic anhydride(10) or alcoholic hydrochloric acid on \( \Delta^{5} \)-cholesten-7-en(11). The structure of \( \Delta^{5,7} \)-cholestadiene was indicated(10) by its method of preparation and from its absorption spectra curve which was analogous with that of ergosterol (the \( \Delta^{5,7} \)-unsaturated derivative of \( \Delta^{5} \)-ergosteren-7-en).
Table I.
Specific Rotations of Various Unsaturated Steroid Derivatives

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<th>Functional groups</th>
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<th>Corrostane</th>
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<td>- Series</td>
<td>- Series</td>
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<td>- Series</td>
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<td>Sat'd</td>
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</table>

*Prepared but no rotation reported.
I and 3 were not included since the preparation of cholestane derivatives unsaturated in rings 6 and 3 were considered of most importance in this investigation.

1. Unsaturated Derivatives of Cholestan-3-ol.

The preparation of derivatives of cholestan-3-ol possessing unsaturation in or adjacent to rings 6 and 3 was made possible by the preparation of 7-dehydrocholesterol in 1935. 7-Dehydrocholesterol is of importance not only as a starting material for the preparation of these derivatives but also because it is the provitamin of vitamin D₂. 7-Dehydrocholesterol is prepared [7] as follows. Chromic acid oxidation of cholesteryl acetate yields 7-ketocholesteryl acetate which is converted to 7-hydroxycholesterol by the action of aluminum isoproxide. Pyrolysis of the dibenzoate of 7-hydroxycholesterol under reduced pressure at 200°C for one and a half hours yields mainly the benzoate of 7-dehydrocholesterol (Δ⁷⁻cholesten-3-ol) [7] and a small amount of Δ⁶⁻cholestan-3-ol benzate [73]. These two isomeric compounds were separated by fractional crystallization of their m-dinitrobenzoates [73].

a. Non-unsaturated Derivatives of Cholestan-3-ol. Sodium-ethyl alcohol reduction [50] of 7-dehydrocholesterol yields Δ⁷⁻cholesterol (Δ⁷⁻cholestan-3-ol). The double bond was indicated to be at the 7-position since treatment of Δ⁷⁻cholesterol with perbenzoic acid was found to yield a cholestanetriol [50]. This cholestanetriol forms a dicarboxylic acid and hence two of the hydroxyl groups were indicated to be secondary and the third hydroxyl group was indicated to be tertiary. Treatment of Δ⁷⁻cholesterol in ethyl acetate solution with platinum catalyst and hydrogen yields Δ⁷⁻cholestenol
\( (\Delta^{(14)} \text{-cholesten}-3\text{-ol}) \) which is also obtained by the catalytic hydrogenation of 7-dehydrocholesterol\(^{(50)}\). The benzoate of \( \text{\Delta}^{(14)} \text{-cholesten}-3\text{-ol} \) is rearranged to the benzoate of \( \text{\Delta}^{(4)} \text{-cholesten}-3\text{-ol} \) by treatment with hydrogen chloride in chloroform solution at \( 0^\circ \) for three hours. The acetate of \( \text{\Delta}^{(4)} \text{-cholestenol} \) is reduced to cholesten-3-ol acetate on catalytic hydrogenation with platinum catalyst in ether-ethyl acetate at room temperature\(^{(50)}\). Sodium-propyl alcohol reduction of \( \Delta^{(3)} \text{-cholestadene}-3\text{-ol} \) was found to give a 70 per cent yield of \( \text{\Delta}^{(5)} \text{-cholesterol} \) \( \Delta^{(4)} \text{-cholesten}-3\text{-ol} \) and a small amount of \( \text{\Delta}^{(4)} \text{-cholestenol} \) of unknown structure\(^{(73)}\). Both \( \Delta^{(5)} \text{-cholestadene}-3\text{-ol} \) and \( \text{\Delta}^{(4)} \text{-cholestenol} \) are converted to \( \text{\Delta}^{(5)} \text{-cholesterol} \) when shaken with ethyl acetate solution with palladium catalyst and hydrogen\(^{(73)}\).

b. Dimethylated Derivatives of Cholesten-3-ol. The benzoate of 7-dehydrocholesterol on treatment with hydrogen chloride in chloroform at \( 0^\circ \) for four hours is converted to the benzoate of dehydrocholesterol \( \Delta_{\text{B}}^{(4,14)} \text{-cholestadene}-3\text{-ol} \)\(^{(50)}\).

The structure of \( \Delta^{(5,6)} \text{-cholestadene}-3\text{-ol} \) is based upon its absorption maximum at 270-280 m\( \lambda \) and its formation of a maleic enhydride addition product. An absorption spectrum maximum at 270-280 m\( \lambda \) indicates a conjugated diene system with both double bonds located in one ring, whereas a maximum at 235-250 m\( \lambda \) indicates that the two double bonds are located in two rings. Furthermore, a maleic enhydride addition product is formed from a conjugated diene system in which the two double bonds are located in the same ring or in two separated rings but not in two adjacent rings.

Normal catalytic hydrogenation of \( \Delta^{(5,6)} \text{-cholestadene}-3\text{-ol} \) with palladium catalyst in the presence of hydrochloric acid yields cholesterol. Treatment with hydrogen chloride in chloroform rearranges \( \Delta^{(5,6)} \text{-cholestadene}-3\text{-ol} \) to
to dehydrocholesterol D\(_5\)(73).

The action of perbenzoic acid on \(\Delta^{11}\)-cholestadiene-3,11-oil at room temperature for four days yields the mono-cetate of \(\Delta^{14}\)-cholestadiene-3,9-diol which is dehydrated by heating with acetic anhydride to \(\Delta^{14}\)-cholestan-3-one acetate (absorption spectra maximum at 283 nm). A compound referred to as cholestadiene-3,6-oil was obtained as a by-product in the treatment of \(\Delta^{14}\)-cholestadiene-3,11-oil with acetic anhydride. No structure was proposed for this compound (possibly \(\Delta^{14}\)-cholestadiene-3,6-diol) although it was found to possess an absorption spectra maximum at 283 nm and it did not form a acetic anhydride addition product(73).

The action of light on a solution of \(\Delta^{14}\)-cholestadiene-3,11-oil in alcohol containing eosin was found to give a 65 per cent yield of a difficultly soluble bimolecular reaction product identical with that formed by a similar treatment of 7-dehydrocholesterol. It has been proposed that this reaction product(3) and the reaction product obtained from \(\Delta^{14}\)-cholestadiene-3,11-oil consist of two steroid nuclei joined to each other at C9 with double bonds located in the 5- and 8(9)-positions(73).

Treatment of \(\Delta^{8}\)-cholesten-3,11-oil with perbenzoic acid yields \(\Delta^{8}\)-cholesten-3,9-diol which on heating with acetic anhydride for two hours is dehydrated to the acetate of cholestadiene-3,11-oil. Cholestadiene-3,11-oil possesses an absorption spectra maximum at 283 nm and is believed to be \(\Delta^{8}\)-cholestadiene-3,11-oil although its specific rotation was not reported(73).

2. Unsaturated Derivatives of Coprosten-3-ol.

a. Mono-unsaturated Derivatives of Coprosten-3-ol. The sodium-cupric alcohol reduction of \(\Delta^{14}\)-coprostan-3,11-oil yields \(\delta\)-coprostenol (\(\Delta^{3}\)-coprostan-3,11-oil). \(\delta\)-Coprostenol is rearranged to \(\gamma\)-coprostenol.
If; 

(\(\Delta^{(14)}\)-coprostan-3-ol) when shaken in ethyl acetate solution with palladium catalyst and hydrogen(76).

b. Unsaturated Derivatives of Coprostan-3-ol. \(\Delta^{(14)}\)-Cholestadiene-3-ol has been found to rearrange to \(\Delta^{(12)}\)-coprostan-3-ol by irradiation with ultraviolet light. The great difference in the specific rotation between these two isomers (see Table I.) is most unexpected since all of the known pairs of corresponding isomers in these two series are quite similar with respect to their specific rotations. The reactions of \(\Delta^{(14)}\)-coprostan-3-ol are analogous with those of \(\Delta^{(12)}\)-cholestadiene-3-ol.

When heated with maleic anhydride it forms an addition product and a small amount of an isomeric compound of unknown structure which has an absorption spectrum maximum at 250\(\mu\)m and might possibly correspond to cholestadienol-3 \(\alpha\). Catalytic reduction of \(\Delta^{(14)}\)-coprostan-3-ol with platinum catalyst in acetic acid at 60\(^\circ\)C in the presence of hydrochloric acid yields coprosterol(76).

\(\Delta^{(12)}\)-Coprostadiene-3-ol acetate is isomerized(76) by the action of hydrogen chloride in chloroform to the acetate of a compound of unknown structure possessing a specific rotation of \(\pm 100^\circ\) \(\alpha\) and an absorption spectrum maximum at 250\(\mu\)m. This compound is converted back to \(\Delta^{(14)}\)-coprostadiene-3-ol when heated with maleic anhydride. Catalytic hydrogenation of this compound with platinum catalyst in acetic acid at 60\(^\circ\)C in the presence of hydrochloric acid yields coprosterol. \(\Delta^{(12)}\)-Coprostadiene-3-ol acetate was found(76) to be photochemically dehydrogenated by exposure in alcoholic solution containing eosin to sunlight to yield a binapthyl compound analogous with that formed by similar treatment of \(\Delta^{(14)}\)- and \(\Delta^{(12)}\)-cholestadiene-3-ol.

a. Mono-unsaturated Ergostane Derivatives. Several mono-unsaturated derivatives of ergostane, ergosten-3-ol, ergosten-3-one and 3-chloroergostane are known.

The known mono-unsaturated derivatives of ergostane are α- and β-ergostane. α-Ergostene (Δ8(14)-ergostene) is obtained by the catalytic hydrogenation of 3-ergostadiene (Δ8(14), 22-ergostadiene), by the sodium-alcohol reduction of 3-chloro-3′-ergostene and by the Clemmensen reduction of 3-ergostene(26). 1-Ergostene has been obtained by the sodium-alcohol reduction of 3-chloro-3′-ergostene and by the Clemmensen reduction of 3-ergostene(27).

The mono-unsaturated derivatives of ergosterol-3-ol include the α-, β- and γ-ergostanol. α-Ergostanol (Δ8(14)-ergostan-3-ol) is obtained by the hydrogenation with either platinum or palladium catalyst of γ-ergostanol(25) and all known ergostan-3-ol derivatives having two or more double bonds. β-Ergostanol (Δ5-ergostan-3-ol) has been prepared by the treatment of either α-ergostanol(27) or 1-ergostanold(27) with hydrogen chloride in chloroform. Formal catalytic hydrogenation of β-ergostanol yields ergostan-3-ol(28). γ-Ergostanol (Δ7-ergostan-3-ol) is obtained by the sodium-alcohol reduction of 22-dihyderoergostanol (Δ8, 7-ergostadiene-3-ol)(70).

Mono-unsaturated derivatives of ergostan-3-one are α- and β-ergostenone. α-Ergostenone (Δ8(14)-ergostan-3-one) has been obtained from α-ergostanol by the action of chromic acid(27) and of copper oxide(25). β-Ergostenone (Δ5-ergostan-3-one) was prepared by treatment of α-ergostanone with hydrogen chloride in chloroform(27) and from β-ergostanol by
the action of copper oxide(27). The 3-chloro derivatives of α- and β-ergosterols were prepared by the action of phosphorus pentachloride on α-ergosterol(27) and β-ergosterol(25).

b. Di-unsaturated Ergostane Derivatives. Since the di-unsaturated ergostane derivatives containing nuclear mono-unsaturation are analogous with the mono-unsaturated ergostane derivatives, they may be considered separately from the nuclear di-unsaturated ergostane derivatives.

The nuclear mono-unsaturated derivatives of ergostane are α- and β-ergostadienes. α-Ergostadiene (Δ^5(16)-ergostadiene) was prepared by the sodium-alcohol reduction of its 3-chloro derivative(26). β-Ergostadiene (Δ^14(18)-ergostadiene) has been obtained by the sodium-alcohol reduction of its 3-chloro derivative and by the treatment of α-ergostadiene with hydrogen chloride in chloroform(26).

The nuclear mono-unsaturated derivatives of ergostan-5-ol are α-dihydro-ergosterol (dihydro-ergosterol I), β-dihydro-ergosterol and dihydro-ergosterol II. α-Dihydro-ergosterol (Δ^5(14),17-ergostadiene-5-ol) is obtained by the hydrogenation of ergosterol with palladium catalyst(53,66), together with α-ergostenol by the hydrogenation of ergosteryl acetate with platinum catalyst(55) and by the sodium-alcohol reduction of ergosteryl acetate, ergosterol peroxide or ergostadienetriol(57,65). β-Dihydro-ergosterol (Δ^14(18)-ergostadiene-5-ol) was prepared from α-dihydro-ergosterol by treatment with hydrogen chloride in chloroform solution(22). Dihydro-ergosterol II (Δ^7(22)-ergostadiene-5-ol) is obtained by the sodium-propyl alcohol reduction of ergosterol or ergosterol I and together with α-α-dihydro-ergosterol I by sodium-alcohol reduction of ergosterol(54,67).

The only known nuclear di-unsaturated derivative of ergostane is
dehydro-ergostene (Δ⁵⁺⁷-ergostadiene) which is obtained by the action of perbenzoic acid on either 5- or 7-ergostene and is converted to ergostene by normal catalytic hydrogenation(70). The nuclear di-unsaturated derivatives of ergostan-7-ol include 3β-dehydroergosterol, dehydro-γ-ergostenol and dehydro-α-ergostenol.

22-Dihydroergosterol (Δ⁵⁺⁷-ergostadiene-3β-ol)(70) is obtained from ergosterol by an indirect method of hydrogenation of the double bond in the side chain of ergosterol without affecting the Δ⁵⁺⁷-diene system of ergosterol. This is accomplished by forming the maleic anhydride addition product of ergosterol acetate to protect the 5,7-unsaturation followed by hydrogenation with palladium catalyst to saturate the 5,7-unsaturation. The maleic anhydride addition product of the 22-dihydroergosterol thus obtained is decomposed by heat treatment at 330°C. A close relationship in specific rotation exists between 22-dihydroergosterol and its derivatives with 7-dehydrocholesterol and its derivatives. The properties and reactions of 22-dihydroergosterol are quite similar to those of ergosterol, even to the point of forming a vitamin D (vitamin D₃).

Dehydro-γ-ergostenol (Δ⁵⁺⁷⁺⁸⁺¹⁰-ergostatetraene-3β-ol) is prepared by the dehydrogenation of γ-ergostenol by the action of mercuric acetate. Dehydro-γ-ergostenol does not add maleic anhydride and may be related to ergosterol χ(70). Dehydro-ε-ergostenol (Δ⁸⁺¹⁰-ergostatetraene-3β-ol) has been obtained by the treatment of ε- or β-ergostanol with perbenzoic acid(70), by the action of bromine on ε-ergostanol(79) and by the treatment of 3β-di-hydroergosterol with hydrogen chloride in chloroform solution(70).

c. Isomers of Ergosterol. The literature on the isomerization of ergosterol is very confusing, due partly to the fact that the structures
of most of the isomers are unknown. However, an understanding of the subject is of importance since it may give an insight on occurrence of steroids in general. The most important of the methods used for isomerization of ergosterol are treatment with hydrogen chloride, hydrogen bromide or dimethyl chloride(1); partial hydrogenation followed by dehydrogenation; dehydrogenation followed by partial hydrogenation. The enantiomers of most of the ergosterol isomers have been prepared by treatment with sodium ethoxide. The perbenzoic acid titration of ergosteryl derivatives is irregular(7).

Treatment of ergosteryl acetate(6) with hydrogen chloride in chloroform solution for one hour at 60° yielded a reaction product which was fractionally crystallized to yield about 20 per cent. of the acetate of ergosterol B, (isosterosterol). The mother liquor from the ergosterol B acetate was treated with maleic anhydride in benene to yield 15 per cent. of an addition product of ergosterol B acetate; the part which did not react with maleic anhydride gave the acetate of ergosterol B. No structures have been proposed for ergosterol B and B, but ergosterol B has been indicated to be ergostatriene-7,14,22-triol(3). Ergosterol B and ergosterol B were shown to have similar absorption spectra and are indicated to have a conjugated system in two rings. Both the B and B isomers can be formed from the B isomer by heating.

Ergosterol was also isomerized with hydrogen chloride(8,9) under apparently the same conditions to obtain isosterosterol (ergosterol(20)). The compound has an absorption maximum at 3.8 m. The structure, 4,16-dimethyl-ergostatriene-7,6,14-triol, has been proposed but this does not seem likely since the isosterosterol yields ergosterol on catalytic reduction, while a compound
having the proposed structure would be expected to yield the expected 
continued.

likewise, a compound(15) has been prepared which we design-
ated as $\Delta^{18,22}$-ergostatriene-3-one, having a melting point of 193-95$^\circ$ and 
a specific rotation of $-56.2^\circ$. This compound was prepared by oxidizing 
the hydroxyl group of ergostanol to a ketone to which we assigned the 
structure of $\Delta^{18,22}$-ergostatriene-3-one. On interaction with alcoholic 
hydrochloric acid, it yielded an isomerization where absorption spectra 
indicated two conjugated double bonds adjacent to a carbonyl group. 
Hydro-
germination of this isomerization by the Weinheim-Fleming method yielded 
an alcohol which is presumably $\Delta^{18,22}$-ergostatriene-3-one.

A product referred to as ergosteral was obtained(15,16) by the 
action of benzoyl chloride on ergostanol, ergosteral B, or ergosteryl B; 
however, it is not considered that this product is of unqualified uniformity.

Ergosteral B is, according to Giller(7), $\Delta^{14,19,22}$-ergostatriene-3-one. 
It can be prepared by the dehydrogenation of o-dihydroergosteryl with 
mercuric acetate(65), or by the action of perchloric acid(77) on selenium 
dioxide on o-dihydroergosteryl. It can also be prepared by the partial 
reduction of dehydroergosteryl ($\Delta^{14,19,22}$, $\Delta^{18,22}$-ergostatriene-3-one) with 
sodium in alcohol(69).

o-Dihydroergosteryl ($\Delta^{14,19,22}$-ergostatriene-3-one) on treatment with 
mercuric acetate was found to yield a product which was referred to as 
ergosteral A (27). Its structure was proposed and doubt has been expressed 
as to whether it is a pure compound(69).

A compound which was referred to as ergosteral(7) (66) was obtained 
by the sodium-alcohol reduction of dehydroergosteryl ($\Delta^{14,19,22}$, $\Delta^{18,22}$-er-
goestatriene-3-one). Sobell(65) has prepared the structure of $\Delta^{18,22}$-er-
ergostatriene-3-ol for this compound. Ergostanol F reacts slowly, if at all, with maleic anhydride(76).

Ergostanol F is isomerized by hydrogen chloride in chloroform to yield ergosterol G and some ergosterol B₁ and B₂(10). Schotter(57) has proposed the structure of Δ⁹⁻[14]₂⁸-ergostatriene-3-ol for this compound, but such a structure seems highly improbable since the product does not react with maleic anhydride, shows no ultraviolet absorption, and is not reduced with sodium in propyl alcohol. Catalytic reduction(40) with platinum catalyst yields 5-ergostanol and ergostanol.

Leuchter(23) points out that the catalytic hydrogenation of ergosterol occurs in two stages: (a) the double bond at C₆-C₇ becomes saturated and the C₈-C₁₀ double bond shifts to C₆-C₁₀ and (b) the double bond in the side chain at C₁₇-C₁₈ becomes saturated yielding α-ergostanol. Dittrich and Ackermann(11) have observed that all ergosterol derivatives having more than one double bond give α-ergostanol as an intermediate product of hydrogenation. Under the usual conditions of catalytic hydrogenation α-ergostanol is almost always the final product; however, if the hydrogenation is performed at elevated temperatures and in the presence of a small amount of hydrochloric acid, the saturated ergostanol is obtained.

4. Other Unsaturated Ergostane Derivatives. Direct evidence in regard to the location of double bonds in compounds of the ergosterol series is lacking in most cases. One exception is the proof of structure of β-ergostanol, which in this case is a question of the location of the double bond. The double bond in α-ergostanol was shown by Ackermann(1) to be at C₁₂-C₁₃; this was later confirmed by Leuchter(23). The evidence for the proposed structure is as follows. Treatment of α-ergostanol acetate with conc
followed by reductive cleavage yielded a non-crystalline neutral fraction which on heating at 170° under reduced pressure yielded a crystalline ketone alcohol, C₃₃H₅₈O₁₀, in which ring B and the side chain are absent, leaving a methyl group at C₁₉ and a ketone group at C₁₆. Selenium dehydrative deamination of the ketone alcohol yielded the known 2-methylphenanthrene.

Further evidence was obtained by distilling the acid fraction of the non-oil under a high vacuum to form a lactone then indicating that the double bond is in a nine-membered ring.

Several hydrocarbons of the ergosterol series have been prepared.

Phenylmethyl-sulphonyl-methyl-ergosterol to form ergostatetraene 1 (49) which has been suggested to have the structure of \( \Delta^{5,7,22} \)-ergostatetraene. Ergostatetraene 1 is converted to ergostatetraene 2 by the action of acetic anhydride (50) or maleic anhydride (51). Ergostatetraene 2 is formed also by the action of \( \beta \)-toluenesulpho-chloride in pyridine on ergosterol (52). No structure has been proposed for the 3-form. The reduction of ergostatetraene 1 with sodium in alcohol yields \( \Delta^{5,7,22} \)-ergostatetraene. Ergostatetraene 2 is not reduced by the action of sodium in alcohol.

\( \Delta^{5,7,22} \)-Ergostatetraene was prepared (10) by oxidizing the hydroxyl group of ergosterol-maleic anhydride to a keto group, reducing the keto group by the Clemmensen method, and then decomposing the maleic anhydride addition product. Further unsaturation in ergosterol was introduced with selenium dioxide (8) and with mercuric acetate (7).

K. Unsatuated 5-β-ol Derivatives.

5-β-Ergosterol is of interest since one of its double bonds has been indicated to be located in ring C. Bernstein and Wallis (3) have investigated
this compound and concluded that the points of unsaturation are at C_6 and C_8(C_14). The compound does not show any absorption maximum and does not add maleic anhydride thus indicating that the double bonds are not conjugated.

Catalytic hydrogenation of the acetate with platinum catalyst at 60° yields \( \Delta^6(14) \)-\( \alpha_1 \)-dihydrositostan-3-ol acetate(\( \alpha_1 \)-dihydrositosteryl acetate), which when isomerized with dry hydrogen chloride in chloroform solution gives \( \alpha_1 \)-isodihydrositosteryl acetate (unsaturated at C_{14}) which on catalytic hydrogenation under ordinary conditions yields \( \alpha_1 \)-sitostan-3-ol acetate. The action of perbenzoic acid on \( \alpha_1 \)-isodihydrositosteryl acetate forms an oxide which on warming in acetic acid containing sulfuric acid is converted to \( \Delta^8,14 \)-\( \alpha_1 \)-sitostadiene-3-ol acetate.

The evidence given by the authors to support the position of the C_6-C_{14} double bond in \( \alpha_1 \)-sitosterol does not seem to eliminate the possibility of the C_8-C_9 position. The possibility of the C_6-C_9 position was claimed to have been eliminated due to the fact that a 7-keto derivative was not obtained when \( \alpha_1 \)-sitosteryl acetate was oxidized with chromic acid. The reason for this deduction is not clear since C_7 is in the \( \alpha \)-position to double bonds both at C_6-C_9 and C_8-C_{14}, and it would seem that one would be as likely as the other to introduce a keto group on carbon number 7.

5. Unsaturated Cholanic Acid Derivatives.

Some of the reactions of the cholic acid series are of interest. Boedecker(5) dehydrated cholic acid by refluxing an acetic acid solution of the compound with zinc chloride for forty-five minutes. Two dehydration products, both involving the removal of the 7-hydroxyl group as water, were obtained. The product obtained in the greater quantity was \( \alpha \)-epocholic
acid (\(\alpha\beta(14)\)-3,12-dihydroxycholenic acid), the structure of which seems to be in little doubt. However, two structures have been proposed for the other dehydration product. According to Wieland the compound is \(\alpha\beta(7)-3,12\)-dihydroxycholenic acid while Callow(7) believes the point of unsaturation is at \(C_{14}\) instead of \(C_7\). The evidence seems definitely to favor Callow's structure. The compound is catalytically reduced with palladium or platinum catalyst to the saturated deoxycholic acid, which would not be expected if it were unsaturated at \(C_7\). Likewise, the formation of \(\alpha\beta(7)\)-dihydroxycholadienic acid and \(\alpha\beta(14)\)-dihydroxycholadienic acid by the action of perbenzoic acid or bromine(6) on the compound in question supports Callow's structure.
Ill. EXPERIMENTAL

3. Cholestenes

1. Preparation of 4\(^{8}\)-Cholestene.

a. Preparation of Cholestane-7-one. Cholestane-7-one was prepared in good yield by the catalytic platinum hydrogenation of 4\(^{8}\)-cholestane-7-one in ethyl acetate solution and also by the reduction of cholestane-7-one with aluminum isopropoxide in isopropanol alcohol solution in a manner similar to the reduction of cholestane(51). Cholestane-7-one was, however, most conveniently prepared by a modification of the sodium-ethyl alcohol procedure of Heilbron, Shaw and Spring(24). Twenty-six grams (26 g.) of sodium in small pieces was added as rapidly as the reaction would permit to a boiling solution of 15 g. of cholestane-7-one in 500 cc. of dry ethyl alcohol in a 1 -liter Frezeneyer flask provided with a reflux condenser. A gentle reflux was continued for a total of three hours so that all of the sodium was consumed. About 400 cc. of water was carefully added with shaking to the cooled solution. The mixture was allowed to stand with occasional gentle shaking until two clear layers formed; the lower water layer was discarded and the upper ethyl alcohol layer was washed once with 100 cc. of water. The solvent was removed from this alcohol solution by distillation in vacuo, and about 100 cc. of water was added near the end of the distillation to facilitate the removal of the last of the ethyl alcohol. The residue was dissolved in ether (about 250 cc.) and water. The ether layer was washed with water and concentrated in vacuo. The residue was dissolved in about 500 cc. of boiling ethyl alcohol and water was
carefully added droplets with stirring to the hot solution until crystallization began. The product was filtered cold to yield 11 gm. of cholestan-7-ol, m.p. 103.107°C.

b. Derivation of Cholestan-7-ol. Cholestan-7-ol was derivatized with anhydrous copper sulfate and with activated alumina under various conditions. In each case a mixture of Δ⁶- and Δ⁶(14)-cholestanes was indicated to be produced, but under certain conditions Δ⁶-cholestane was produced in a much higher proportion than the other thus making it practicable to prepare it in relatively good yield.

The procedure which was found best for the preparation of Δ⁶-cholestene is as follows. A mixture of 6 gm. of cholestan-7-ol, 8 gm. of finely powdered anhydrous copper sulfate, 50 cc. of xylene (technical or pure), and 0.2 cc. of propionic acid in a 200 cc. Erlenmeyer flask fitted with a reflux condenser was refluxed for five hours. The reaction mixture was cooled; about 60 cc. of petroleum ether (b.p. 30 to 40°C) was added and the solution was decanted through a 1.0 x 30 cm. column of activated (freshly heated at 200°C for two hours) alumina (30 to 200 mesh Harco). The flask was washed with two 15 cc. portions of solvent and the washings were also passed through the column. The alumina column was washed with about 200 cc. of petroleum ether. The combined filtrates were concentrated under the reduced pressure of a water pump and the residue was dissolved in about 200 cc. of hot acetone. Hot methanol was carefully added to the point of turbidity whereupon well formed white plates crystallized. After standing for several hours in a refrigerator, the material was filtered to yield 3.0 gm. of plates, m.p. 70-77°C, which after ten recrystallizations from acetone-methanol yielded Δ⁶-cholesterol, m.p. 35-36°C, (α)₂₅°[]₁₁.₃° (c, 3.5) in carbon tetrachloride.
The alumina column was eluted with about 100 cc. of ether and the
eluate was concentrated in vacuo. The residue was dissolved in hot acetone
and water was carefully added with stirring until crystallization began.
The material was cooled in a refrigerator for several hours and then filtered
to yield 1.1 gm. of unchanged cholestan-7-ol, m.p. 116-118°.

The procedures which were found to yield a mixture of \( \Delta^5 \) and \( \Delta^{(14)} \)
cholestan-7-ol are as follows. In each case the hydrocarbon fraction was
separated from unchanged cholestan-7-ol as previously described. A mixture
of 5.0 gm. of cholestan-7-ol, 5.0 gm. of sodium copper sulfate
and 30 cc. of xylene (reagent grade) was refluxed four hours. A mixture
of cholesatan-7-ol weighing 1.2 gm. was obtained from the reaction product,
thus indicating that most of the cholestan-7-ol had been dehydrated. By
a tedious process of fractional crystallization from acetone, \( \Delta^5 \) and
\( \Delta^{(14)} \)-cholestan-7-ol were obtained; the \( \Delta^{(14)} \)-cholestan-7-ol crystallized in
the form of needles, m.p. 67-68°.

An intimate mixture of 1 gm. of cholestan-7-ol and 1 gm. of powdered
anhydrous copper sulfate was heated at 155-160° under the reduced pressure
produced by a water pump for one hour to yield 0.3 gm. of a mixture of
\( \Delta^5 \) and \( \Delta^{(14)} \)-cholestan-7-ol.

A mixture of 1 gm. of cholestan-7-ol, 1 gm. of anhydrous copper sulfate
and 10 cc. of toluene containing 0.1 cc. of acetic acid was refluxed four
hours to yield 0.2 gm. of crude \( \Delta^5 \)-cholestan, m.p. 75-79°.

A mixture of 0.5 gm. of cholestan-7-ol and 0.5 gm. of activated alumina
(200 mesh) was heated under a pressure of approximately 1 mm. of mercury,
beginning at 160° then increasing the temperature over a period of ten
minutes to 260-70° where it was held for an additional fifteen minutes. The reaction product yielded 35 mg. of crude \( \Delta^6 \)-cholestene, m.p. 74-80°.

The foregoing reaction was repeated except that the reaction was carried out at atmospheric pressure under nitrogen at 290-5° for forty-five minutes. The reaction product yielded 0.22 gm. of a hydrocarbon product which on slow crystallization from acetone-methanol yielded a mixture of plates, m.p. 72-8° (impure \( \Delta^6 \)-cholestene) and needles, m.p. 49-53° (impure \( \Delta^6(14) \)-cholestene).

c. Conversion of \( \Delta^6 \)-Cholestene to Cholestan-7-one. A mixture of 10 gm. of \( \Delta^6 \)-cholestene, 200 cc. of benzene, 60 cc. of acetic acid, and 10 gm. of chromic anhydride in 150 cc. of dilute sulfuric acid (1:3) was stirred vigorously for six hours at room temperature (23° to 27°). Alcohol (5 cc.) was added, the benzene layer was removed, and the sulfuric acid layer was extracted once with 200 cc. of ether. The combined benzene and ether solutions were washed with water, then with 5 per cent sodium hydroxide and finally with water. The solution was dried over sodium sulfate and concentrated in vacuo. The residue dissolved in 100 cc. of petroleum ether was passed through an 13 x 240 mm. column of activated alumina (30 to 200 mesh). The column was washed with 200 cc. of petroleum ether; the combined filtrates were concentrated in vacuo and the residue was crystallized from acetone-methanol to yield 2.8 gm. of unchanged \( \Delta^6 \)-cholestene, m.p. 34-5°, which was indicated by absorption spectrum data to be slightly contaminated with a cholestenadiene.

The alumina column was eluted with 600 cc. of ether. The ether eluate was concentrated in vacuo to yield 4.2 gm. of an oil which on slow crystallization from methanol yielded 1.1 gm. of a ketone in the form of plates, m.p. 61-6°, which after several recrystallizations from methanol melted at 86.5-7.5°, (c) \( \Delta^21 \) 51.8° (c, 2.15 in COCl). An absorption maximum at 251 μm indicated that this ketone is a cholestenone. Although this cholestenone was definitely obtained several times, many unsuccessful attempts were probably due to impurities in the \( \Delta^6 \)-cholestene.

*Anal.* Calc'd. for C_{29}H_{40}: C, 84.30; H, 11.55. Found: C, 84.06, 83.95;
The solvent of the ketone filtrate was removed by concentration with warming in vacuo and its analysis indicated that this remainder of the ketone fraction was essentially only diketones. A diketone was isolated from certain reaction products in the form of needles which melted at 74-5° and (α)D was -53.3° (c, 1.30 in carbon tetrachloride).

Anal. Calc'd. for C_{20}H_{36}O_2: C, 80.93; H, 11.08. Found: C, 81.17; H, 10.32 for the diketone fraction. Found: C, 81.17, 81.25; H, 10.68, 10.77 for the diketone.

Two grams of sodium was added over a period of two hours to a boiling solution of 500 mg. of the choleseterone in 50 cc. of amyl alcohol. Fifteen cubic centimeters (15 cc.) of water was added and, after allowing to stand for one hour with occasional shaking, the water layer was discarded. One hundred cubic centimeters (100 cc.) of ether was added to the alcohol solution, and the solution was washed with water and concentrated in vacuo. The residue was dissolved in hot methanol and, on slow cooling with the the dropwise addition of water to turbidity, 310 mg. of a nonhomogeneous semicrystalline product was obtained. This product was then oxidized with chromic acid, using the same procedure as was applied to the oxidation of 4α-cholestone, to yield 190 mg. of plates which after several recrystallizations melted at 115-6° and formed an oxine which melted at 135-5°.

This ketone did not add bromine and is therefore indicated to be a choles-
tanone. A mixed melting point of 114-6° was observed when the choles-tanone was mixed with cholestan-7-one, m.p. 113-4° (purified by a mild chromic
acid oxidation and subsequent crystallizations). Also, the oxime of the cholestanone did not depress the mixed melting point of the oxime. m.p. 13Î/2°, of cholestan-7-one; therefore the cholestanone is indicated to be identical with cholestan-7-one.

2. Preparation of Δ9(14)-Cholesten.

Although Δ9(14)-cholestene was isolated from certain of the dehydration products of cholestan-7-al, it can be prepared much more conveniently by shaking Δ8-cholestene with palladium catalyst and hydrogen. It is not necessary to use pure Δ8-cholestene since the mixture of Δ8- and Δ9(14)-cholestenes as obtained by the dehydration of cholestan-7-al, can be almost quantitatively converted to Δ9(14)-cholestene.

A solution of 3 gm. of Δ8-cholestene (pure or crude) in 71 gm. of ethyl acetone was shaken with hydrogen and 6.2 gm. of palladium catalyst for three hours. The palladium was removed by filtration and the ethyl acetate was removed by concentration in vacuo. The residue was dissolved in hot acetone and crystallized by cooling in a refrigerator. After several hours an equal volume of methanol was added and the product was finally cooled in a freezing bath before filtration to yield 2.7 gm. of Δ9(14)-cholesten in needles, m.p. 53-55°, which after repeated recrystallizations from acetone-methanol, m.p. at 53-54° (c. 3.17 in carbon tetrachloride).

3. Preparation of $\Delta^\{14\}$-Cholestene.

- Treatment of $\Delta^\{14\}$-Cholestene with Hydrogen Chloride. Dry hydrogen chloride was passed through a solution of 2.5 cc. of $\Delta^\{14\}$-cholestene in 20 cc. of chloroform at 0° for three hours. The solvent was removed in vacuo and the residue was dissolved in 75 cc. of ether. The ether solution was placed in a separatory funnel and shaken vigorously with 10 per cent sodium hydroxide for ten minutes. The ether layer was washed with water and dried over sodium hydroxide pellets. The ether was removed in vacuo, and the residue was dissolved in about 25 cc. of petroleum ether (Shelly 1) and passed through a 6 x 70 cm. column of activated alumina (3-20 mesh). The column was rinsed with 25 cc. of petroleum ether, and the combined filtrates were concentrated in vacuo. The residue was crystallized by dissolving it in about 55 cc. of acetone, cooling in a freezing bath and adding an equal volume of methanol over a period of one hour. Recrystallization yielded 0.21 gm. of $\Delta^\{14\}$-cholestene in the form of plates which after numerous recrystallizations from acetone-methanol melted at 75-76° and $(c)_{D}^{22}$ was +26.6° (c, 3.05 in carbon tetrachloride).

*Anal. Calc.* For Cholest: C, 77.17; H, 12.53. Found: C, 77.06; 77.28; H, 12.50, 12.67.

The alumina column was eluted with 50 cc. of ether and the filtrate was concentrated in vacuo. The residue was dissolved in 10 cc. of acetone; 5 cc. of methanol was added and the solution was allowed to evaporate slowly over a period of several days to yield 30 cc. of a compound in the form of needles which after several recrystallizations from acetone melted at 113-115° and $(c)_{D}^{22}$ was +37.1° (c, 1.33 in carbon tetrachloride).
compound was halogen free, did not react with bromine, was found to be unchanged by refluxing one hour with alcoholic hydrochloric acid and was strongly adsorbed by activated alumina. The properties of the compound together with its analysis indicate the compound to be a cholesterol.

Anal. Calcd. for C_{30}H_{48}O: C, 83.5%; H, 12.6%. Found: C, 83.6%; H, 12.4%.

b. Treatment of Δ^{2}-Cholestene with Hydrogen Chloride. Dry hydrogen chloride was passed through a solution of 0.5 gm. of Δ^{2}-cholestene in 20 cc. of chloroform at 0° for two hours. The product was worked up in the same manner as applied to a similar treatment of Δ^{14}-cholestene. The column filtrate yielded 0.15 gm. of Δ^{14}-cholestene in the form of plates which after several recrystallizations melted at 73-75°.

c. Catalytic Hydrogenation of Δ^{14}-Cholestene. A mixture of 50 cc. of Δ^{14}-cholestene, 50 cc. of ethyl acetate and 100 mg. of platinum oxide was shaken with hydrogen under 20 pounds pressure at room temperature for six hours. The platinum was removed by filtration and the filtrate was concentrated in vacuo. The residue was dissolved in 10 cc. of carbon tetrachloride and the solution was transferred to a 125 cc. separatory funnel. Twenty cubic centimeters (20 cc.) of acetic anhydride was added and then with shaking and cooling, 30 drops of concentrated sulfuric acid was added dropwise through the stem of the separatory funnel. The resulting solution was allowed to stand for twenty minutes and then 3 cc. of water was added with shaking and cooling through the stem of the separatory funnel. The lower layer was removed and washed several times with water. The solvent was removed in vacuo and the residue was dissolved in 15 cc. of petroleum ether. The solution was passed through an 8 x 21 cm. column of activated
alumina and the column was washed with 20 cc. of petroleum ether. The combined filtrates were concentrated in vacuo and the residue was crystallized from 30 cc. of absolute alcohol to yield 29 mg. of a compound, m.p. 79-80°, which gave no depression in mixed melting point with an authentic sample of cholestene.

4. Bromine and Perbenzoic Acid Titrations of Cholestenes

a. Titration with Bromine. A chloroform or methanol solution of bromine cooled to 0° was added to a chloroform or methanol, respectively, solution of each compound cooled to 0°. The amount of bromine added was that amount which was previously found to be in slight excess of that amount consumed by the compound during five minutes at 0°. After standing for five minutes at 0°, a solution of about 0.3 gm. of potassium iodide dissolved in 10 cc. of dilute acetic acid (5 per cent) was added to each titration mixture and the liberated iodine was titrated with 0.1 N sodium thiosulfate solution. The amount of bromine in molar equivalents consumed by each compound is shown in Table II.

Table II.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chloroform solutions</th>
<th>Methanol solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ⁵-Cholestene</td>
<td>1.02</td>
<td>1.07</td>
</tr>
<tr>
<td>Δ⁶-Cholestene</td>
<td>3.00</td>
<td>1.71</td>
</tr>
<tr>
<td>Δ⁸(¹⁴)-Cholestene</td>
<td>3.37</td>
<td>2.15</td>
</tr>
<tr>
<td>Δ¹⁵-Cholestene</td>
<td>2.92</td>
<td>1.31</td>
</tr>
</tbody>
</table>
b. Titration with Perbenzoic Acid. A chloroform solution of perbenzoic acid (approximately three atom equivalents of oxygen) was added to 100 mg. samples of the cholestanones dissolved in 5 cc. of chloroform. The samples together with blanks were kept in a refrigerator for seven days and then the excess perbenzoic acid was titrated with 0.1 m sodium thiosulphate after the addition of a solution of potassium iodide containing acetic acid. 

Δ⁵-cholestanone, Δ⁶-cholestanone, Δ²(16)-cholestanone and Δ¹⁰-cholestanone absorbed 4.15, 9.18, 3.18 and 7.53 mg. of oxygen which corresponds to 1.83, 2.11, 1.69 and 1.81 atom equivalents of oxygen respectively.

D. Cholestanones

I. Characterization of Δ⁶,⁶-Cholestanones.

a. Preparation of Δ⁶,⁶-Cholestanones. The preparation of Δ⁶,⁶-cholestanones by two methods, the dehydration of Δ⁶-cholesten-7-ol and the ketone reduction of Δ⁴,⁶-cholestanol-7-one, was investigated. Δ⁶-Cholesten-7-ol was dehydrated as follows. An intimate mixture of 0.5 gm. of Δ⁶-cholesten-7-ol and 0.5 gm. of activated alumina (200 mesh) was heated at 250–50° for two hours. The reaction product was extracted with 55 cc. of petroleum ether and passed through on 1.5 x 40 cm. column of activated alumina (50 to 200 mesh). The column was washed with 50 cc. of petroleum ether and the combined filtrates were concentrated in vacuo. The residue was crystallized from alcohol to yield 95 mg. of needles, m.p. 83–7°, which after four recrystallizations from alcohol melted at 83–7° and (Δ)²⁶ = +1.65° (c, 2.77 in carbon tetrachloride). The product gave no depression in mixed melting point with a sample of 7-dehydrocholestanone isomer which was obtained by
the treatment of $\Delta^5$-cholesten-7-one with alcoholic hydrochloric acid (14).

An intimate mixture of 0.3 gm. of $\Delta^5$-cholesten-7-one and 0.1 gm. of activated alumina (200 mesh) was heated at 295-305$^\circ$ for one hour. The de-
hydration product was worked up in a manner similar to that described above
to yield 0.1 gm. of a product which after repeated recrystallizations melted
at 74-76$^\circ$ and $\delta_D^{b}$ was $-36.0^\circ$. A solution of 50 mg. of this product in
15 cc. of alcohol containing 1 drop of concentrated hydrochloric acid was
refluxed for two hours and the product recovered melted at 75-77$^\circ$ and $\delta_D^{b}$
was $-30.5^\circ$.

A mixture of 0.2 gm. of $\Delta^5$-cholesten-7-one, 0.3 gm. of suberic copper
sulfate and 6 cc. of xylene was refluxed for eight hours. To the reaction
mixture was added 30 cc. of petroleum ether and this was decanted through
an 18 x 25 mm. column of activated alumina. The column was washed with 30 cc.
of petroleum ether and the combined filtrates were concentrated in vacuo.
The residue was crystallized from alcohol to yield 0.13 gm. of needles which
melted at 80-82$^\circ$ and $\delta_D^{b}$ was $-5.5^\circ$ (c. 2.00 in carbon tetrachloride).

$\Delta^4$-cholestan-3-one was reduced as follows. A mixture of 2.8 gm.
of the semicarbazone of $\Delta^6$-cholestadiene-3-one and 2.5 gm. of sodium in
26 cc. of alcohol was heated in a sealed tube at 300$^\circ$ for eight hours. The
reaction product was dissolved in ether; the other solution was washed with
water, dried over sodium sulfate and concentrated in vacuo. The residue was
dissolved in 30 cc. of petroleum ether and passed through an 18 x 100 mm.
column of activated alumina; the combined filtrates were concentrated in vacuo
and the residue was crystallized from acetone-ether to yield 1.1 gm. of
needles, m.p. 77-79$^\circ$, $\delta_D^{b}$-7.6$^\circ$ (c. 2.15 in carbon tetrachloride). The
melting point and optical rotation gradually changed during the course of
Twenty-three recrystallizations from acetone-methanol to yield a product which melted at 82.5-83°C and (α) D 25° was -35.1° (c, 2.15 in carbon tetrachloride).

b. Cholic Acid Estimations. "7'-Dehydrocholestan-7-one", which was prepared by the action of alcoholic hydrochloric acid on δ-cholesten-7-one (14), was oxidized with chronic acid according to the action of Fentl (15) to prepare δ-cholesten-3,6-dione from cholesteriine. A mixture of 3.5 g. of "7'-dehydrocholestan-7-one", 70 cc. of benzene, 30 cc. of glacial acetic acid, and 4.3 g. of chronic anhydride in 56 cc. of dilute sulfuric acid (1:3) in a 250 cc. round bottom flask was stirred mechanically for six hours at room temperature. At the end of this time alcohol was added to destroy the excess chronic acid. The resulting mixture was transferred to a 500 cc. separatory funnel, about 100 cc. of water was added, the benzene layer was separated and the water layer was extracted with two 75 cc. portions of ether. The benzene layer combined with the ether extracts was washed several times with 5 per cent sodium hydroxide and water, dried over anhydrous sodium sulfate and concentrated by distillation in vacuo. The residue was crystallized from 30 cc. of ether in a freezing bath and filtered to yield 0.75 gm. of a cholestanedione in pale yellow plates, m.p. 150-7°C, which after several recrystallizations from alcohol melted at 160-1°C and (α) D 25° was -51.7° (c, 1.02 in carbon tetrachloride). The diacemicabenzene melted at 328°C with decomposition.


The cholestanedione from "7'-dehydrocholestan-7-one" was reduced as follows. The diacemicabenzene (0.15 gm.) of the cholestanedione was heated with a solution of 0.50 gm. of sodium in 9 cc. of absolute alcohol and 0.5 cc.
of 0.0 per cent hydrochloric hydrate in a sealed tube at 200°C for eight hours. The reaction mixture was dissolved in ether and the ether solution was washed with water, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was dissolved in 20 cc. of petroleum ether and passed through a 9 x 30 cm. column of activated alumina. The column was washed with 50 cc. of petroleum ether and the combined filtrates were concentrated in vacuo. The residue was crystallized from alcohol to yield 0.0 g. of needles, m.p. 79-80°, which after seven recrystallizations from alcohol melted at 78-80°. The mixed melting point with an authentic sample of coprostene (m.p. 77-78°) was 78-80°, indicating identity of the two samples. The identity was further verified by brominating the hydrocarbon to yield plates, m.p. 119-120°, which melted at 119-120° when mixed with an authentic sample of coprostene dibromide, m.p. 123-124°.

The hydrocarbon obtained by the reduction of 7α,8α-cholestadiene-3-one was oxidized as follows. A mixture of 0.60 g. of the hydrocarbon, 1.2 cc. of benzene, 5 cc. of acetic acid, and 0.33 g. of chromic anhydride in 9 cc. of dilute sulfuric acid (1:3) was stirred mechanically for six hours at room temperature. The benzene layer was separated and the remaining liquid was extracted with two 50 cc. portions of ether. The ether extracts were added to the benzene layer and the whole then washed several times with dilute sodium hydroxide and water. The solution was dried over anhydrous sodium sulfate, the solvent removed by distillation under the reduced pressure of a water pump, and the residue crystallized from 3 cc. of ether and 1 cc. of methanol to yield 15 mg. of an amorphous solid, m.p. 105-106°, which after two recrystallizations from ether-methanol yielded plates, m.p. 157-158°. The mixed melting point with a sample of the cholestadiene
(m.p. 158-9\(^\circ\)) obtained by a similar oxidation of the "7-dehydrocholestone isomer" was 157-9\(^\circ\) which indicated the identity of the two compounds.

c. Catalytic hydrogenation of "7-Dehydrocholestone Isomer". A solution of 0.75 gm. of "7-dehydrocholestone isomer" in 50 cc. of ethyl acetate was shaken overnight with hydrogen in the presence of 0.1 gm. of palladium oxide catalyst. The reduction product was filtered to remove the palladium and the solvent was removed by distillation in vacuo. The residue was dissolved in 50 cc. of carbon tetrachloride. This solution was transferred to a separatory funnel, 25 cc. of acetic anhydride was added and then 3 cc. of concentrated sulfuric acid was added dropwise through the stem of the funnel with shaking and cooling under a water top. After standing twenty minutes, 3 cc. of water was added dropwise with cooling through the stem of the funnel. The top acid layer was discarded. The carbon tetrachloride layer was washed with sodium bicarbonate solution and water, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was dissolved in hot alcohol, treated with decolorizing carbon and filtered hot.

By means of fractional crystallization, cholestanone, m.p. 79-80\(^\circ\), in the form of plates, and coprostone, m.p. 68-9\(^\circ\), in the form of needles, were obtained. The proportion of cholestanone to coprostone isolated was 0.8 to 1.

g. Treatment of "7-Dehydrocholestone Isomer with Hydrogen Chloride.

Dry hydrogen chloride was passed through a solution of 300 mg. of "7-dehydrocholestone isomer" in 25 cc. of chloroform (dried over phosphorus pentoxide and distilled) at 0\(^\circ\) for three hours. The solvent was removed in vacuo at room temperature and the residue was dissolved in 75 cc. of petroleum ether (b.p. 35-40\(^\circ\)) was passed through an 18 x 65 cc. column of activated alumina (30 to 200 mesh preheated Marco). The column was washed
with 50 cc. of petroleum ether and the combined filtrates were concentrated in vacuo. The residue on one crystallization from absolute alcohol yielded 190 mg. of \( \Delta^5_6 \)-cholestenol, m.p. 73.5-79\(^\circ\), (c) \(-103.8\) (c, 1.25 in carbon tetrachloride).

The eluting column was eluted with 100 cc. of ether and the eluate was concentrated in vacuo. The residue was crystallized from petroleum ether to yield 50 mg. of a halogen-free solid which after several recrystallizations from acetone gave a compound in the form of needles, m.p. 160-170\(^\circ\), (c) \(+54\) (c, 1.25 in carbon tetrachloride).


This compound was found to add bromine and to form a complex, m.p. 62-63\(^\circ\). Dehydration with alcoholic hydrochloric acid was found to convert the compound to \( \Delta^5_6 \)-cholestenol, m.p. 73.5-79\(^\circ\), (c) \(-111.7\) (c, 1.35 in carbon tetrachloride). The compound gave no depression in mixed melting point with an authentic sample of the molecular compound of allic- and spi-alfa-cholesterol (m.p. 140-141\(^\circ\)) and was separated by digitonin into a compound, m.p. 131-132\(^\circ\), which gave no depression in mixed melting point with an authentic sample of allic-cholesterol, and a compound, m.p. 52-53\(^\circ\), which gave no depression in mixed melting point with an authentic sample of spi-alfa-cholesterol.

e. **Examination of the 5,6-Dibromcholestanone-quinine Product.**

A slight excess of bromine in glacial acetic acid was added to a solution of 25 mg. of the 5,6-Dibromcholestanone-quinine product dissolved in ether. After standing for two minutes, \( \frac{1}{4} \) drops of acetone was added and the solution was allowed to stand in a refrigerator for several days until crystals
for a which melted at 113-6°C and gave a mixed melting point of 115-6°C
with an authentic sample of \( \Delta^2 \)-cholestanol (cholesterol, m.p. 115-6°C).

2. Preparation of \( \Delta^7,\Delta^{11} \)-Cholestadiene.

Bromine (1.05 mole) in 15 cc. of dry chloroform at -70 to -50°C was
added all at once to a solution of 1.5 gms. of \( \Delta^7 \)-cholestanol in 100 cc.
of dry chloroform at approximately -70°C. The solvent was removed in vacuo;
the residue was taken up in 50 cc. of petroleum ether (Shelly I) and passed
through an 16 x 70 mm. column of activated alumina. The column was rinsed
with 50 cc. of petroleum ether, and the combined filtrates were concentra-
ted in vacuo. The residue was crystallized from acetone-methanol to yield
1.03 gms. of plates, m.p. 75-82°C, which after several recrystallizations
from acetone-methanol, or treatment with dry hydrogen chloride at 0°C for
two hours and working up, melted at 83-4°C, \( \varepsilon \) = 3530 cm\(^{-1}\) (c, 1.96 in carbon
tetrachloride). An absorption maximum was observed at 283 m\(\nu \) (0.2 mg. per
cc. in alcohol).

Incl. Calcd. for C\(_{27}\)H\(_{44}\): C, 87.96; H, 12.04. Found: C, 87.65, 87.76;
H, 12.14, 12.21.

3. Preparation of \( \Delta^7,\Delta^{11} \)-Cholestadiene.

A solution of 2 gms. of \( \Delta^7 \)-cholestanol and twice the calculated amount
of perchloric acid in 20 cc. of chloroform was allowed to stand in a re-
frigerator for eight days. The solvent was removed in vacuo at room tempera-
ture and the residue was dissolved in 100 cc. of ether; the ether solution
was washed with 5 per cent sodium hydroxide solution and water, and then
dried over anhydrous sodium sulfate. The solvent was removed in vacuo.
and the residue was dissolved in 75 cc. of petroleum ether and passed through an 18 x 190 mm. column of activated alumina (30 to 60 mesh). The column was washed with 75 cc. of petroleum ether; the combined filtrates were concentrated in vacuo, and the residue was crystallized from acetone-methanol to yield 0.6 gm. of thick plates, m.p. 52-53°C, (c)\textsubscript{D}\textsuperscript{-75°} 25°C (c, 1.27 in carbon tetrachloride). Absorption maxima at 250nm and 282nm were observed (0.04 mg. per cc. in alcohol). An attempt to purify the product further by recrystallization (six times) from acetone-methanol gave a product, m.p. 76-80°C, (c)\textsubscript{D}\textsuperscript{-78.1°} 25°C (c, 2.03 in carbon tetrachloride).

**Anal.** Calcd. for C\textsubscript{30}H\textsubscript{48}O: C, 87.96; H, 12.04. Found: C, 87.59, 87.71; H, 12.13, 12.02.

The alumina column was eluted with 300 cc. of ether and the eluate was concentrated in vacuo. Attempts to crystallize the residue from various solvents were unsuccessful. The residue was dissolved in 50 cc. of methanol containing 10 drops of dilute sulfuric acid (1:1) and the solution was refluxed for twenty minutes. The solution was neutralized with a few drops of concentrated sodium hydroxide solution. The solvent was removed in vacuo and the residue was dissolved in ether, washed, dried and concentrated in vacuo. The residue was dissolved in about 20 cc. of hot petroleum ether (Shelly B) and the solution was gradually cooled in a freezing bath to yield on filtration 0.25 gm. of an amorphous solid. After four recrystallizations from petroleum ether the compound was obtained in plates, m.p. 220-1°C, (c)\textsubscript{D}\textsuperscript{+73°} 50.5°C (c, 0.12 in carbon tetrachloride). This compound was indicated to be a cholestanediol by its analysis.

**Anal.** Calcd. for C\textsubscript{30}H\textsubscript{48}O: C, 80.17; H, 11.96. Found: C, 80.12, 80.36; H, 11.81, 11.93.
An attempt was made to acetylate the diol. A solution of 100 mg. of the diol, 1 cc. of acetic anhydride, and 2 cc. of pyridine was allowed to stand at room temperature for twenty-four hours. On working up the reaction product 35 mg. of plates, m.p. 199-200°C, were obtained which did not depress the melting point of the starting material.

A solution of 100 mg. of the diol in 5 cc. of acetic anhydride was baked at 100°C for one hour. On cooling 38 mg. of plates, m.p. 199-200°C, were obtained which did not depress the melting point of the starting material.

A solution of 28.2 mg. of the diol in 5 cc. of a saturated solution of lead tetraacetate in acetic acid was allowed to stand at room temperature for twenty hours and then the excess lead tetraacetate was titrated according to Griege. The sample required 7.68 cc. of 0.1 N sodium thiosulfate while a blank required 7.43 cc. of thiosulfate thus indicating that no oxidation occurred.

4. Preparation of \( \Delta^8,14 \)-Cholesterol.

a. Treatment of \( \Delta^8(14) \)-Cholestene with Perbenzoic Acid. A mixture of 1 gm. of \( \Delta^8(14) \)-cholestene and 0.75 gm. of perbenzoic acid (two moles per mole of \( \Delta^8(14) \)-cholestene) dissolved in 30 cc. of chloroform was allowed to stand stoppered at 0°C for eight days. The reaction product was washed with 5 per cent sodium hydroxide and with water, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue dissolved in 100 cc. of methanol containing 1 cc. of 15 N sulfuric acid was refluxed for fifteen minutes. The solution was neutralized with 35 per cent sodium hydroxide and concentrated in vacuo. The residue was extracted with
75 cc. of petroleum ether and the extract was passed through an 13 x 170 mm. column of activated alumina. The column was washed with 150 cc. of petroleum ether and the combined filtrates were concentrated in vacuo. The residue was crystallized from acetone-methanol and the 150 mg. of plates on repeated recrystallization yielded $\Delta^5,14$-cholestenone, m.p. 37-39$^\circ$, (c) $^{25}$C-37.0$^\circ$ (c, 1.06 in carbon tetrachloride), which gave no depression in mixed melting point with a sample of $\Delta^5,14$-cholestenone obtained by treatment of $\Delta^5$-cholesterol with selenium dioxide.

b. Treatment of $\Delta^{2(14)}$-Cholesterol with Selenium Dioxide. A mixture of 200 mg. of $\Delta^{2(14)}$-cholesterol, 200 mg. of selenium dioxide, and 10 cc. of alcohol was refluxed for six hours. The solvent was removed in vacuo and 25 cc. of petroleum ether was added. The petroleum ether solution was passed through an 13 x 120 mm. column of activated alumina. The column was washed with 40 cc. of petroleum ether and the combined filtrates were concentrated in vacuo. The residue was crystallized from alcohol to yield 30 mg. of product in the form of plates, m.p. 73-75$^\circ$. After six recrystallizations from alcohol, the product melted at 73-75$^\circ$, (c) $^{25}$C-19.7$^\circ$ (c, 1.06 in carbon tetrachloride) and gave no depression in mixed melting point with $\Delta^5,14$-cholestenone obtained by the treatment of $\Delta^{2(14)}$-cholesterol with perbenzoic acid.

Found: C, 87.56; H, 12.02. Calcd. for C$_{27}$H$_{44}$O: C, 87.56; H, 12.42.

o. Treatment of $\Delta^{2(14)}$-Cholesterol with Bromine. Three mols of bromine in methanol was added to a solution of 200 mg. of $\Delta^{2(14)}$-cholesterol in 75 cc. of methanol-ether (1:1). The mixture was allowed to stand in a refrigerator for one day, then the solvent was removed and the residue
was extracted with petroleum ether. The petroleum ether extract was then passed through an 8 x 50 cm. column of activated alumina. The filtrate was evaporated and the residue was crystallized from acetone-methanol to yield 18 mg. of plates, m.p. 83-85° which after two recrystallizations from acetone-methanol, melted at 82-83°, (α)30°-15° (c. 1.20 in carbon tetrachloride) and was not depressed in melting point when mixed with Δ6,14-cholestadiene.

d. Treatment of Δ6(14)-Cholestane with Chronic Acid. Δ6(14)-Cholestene (0.3 gm.) was oxidized with chronic acid in the same manner as applied to Δ6-cholestene. The product was worked up in a similar manner to yield a ketone fraction and a hydrocarbon fraction. The ketone fraction could not be crystallized either directly or through the semicarbazone or oxime, but the hydrocarbon fraction yielded 12 mg. of plates, m.p. 83-85° which caused no depression in melting point when mixed with Δ6,14-cholestadiene.

e. Catalytic Hydrogenation of Δ6,14-Cholestadiene. A mixture of 50 mg. of Δ6,14-cholestadiene, 50 cc. of ethyl acetate and 200 mg. of palladium oxide was shaken with hydrogen at room temperature under 30 pounds pressure for ten hours. The palladium was removed by filtration and the filtrate was concentrated in vacuo. The residue was crystallized in a freezing bath from acetone-methanol to yield 33 mg. of pure product in the form of needles, m.p. 150-151°. After three recrystallizations from acetone-methanol, the product melted at 155-156° and gave no depression in mixed melting point with Δ6(14)-cholestene.

f. Treatment of Δ6,14-Cholestadiene with Maleic Anhydride. A solution of 50 mg. of Δ6,14-cholestadiene, 20 mg. of maleic anhydride and 30 cc. of dry benzene was refluxed for nine hours. About 50 cc. of ether was added
and the resulting solution was washed several times with water and dried over anhydrous sodium sulfate. The solvent was removed in vacuo and the residue on crystallization from acetone-n-butanol yielded 3.5 g. of product in the form of plates. On recrystallization from acetone-n-butanol the product melted at 63-65° and gave no depression in mixed melting point with the original $\Delta^{8,14}$-cholestanone.

5. Bromine Titrations of Cholestadienes.

The cholestadienes, including $\Delta^{8,14}$-cholestanone as a check, were titrated with bromine in the same manner as applied to the cholestanes (see page 36).

Table III.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chloroform solutions</th>
<th>Methanol solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta^{8,14}$-Cholestanone</td>
<td>1.61</td>
<td>1.65</td>
</tr>
<tr>
<td>$\Delta^{8,5}(11)$-Cholestanone</td>
<td>3.00</td>
<td>1.65</td>
</tr>
<tr>
<td>$\Delta^{8,13}$-Cholestanone</td>
<td>3.66</td>
<td>1.76</td>
</tr>
<tr>
<td>$\Delta^{8,14}$-Cholestanone</td>
<td>3.38</td>
<td>1.85</td>
</tr>
</tbody>
</table>

6. Cholestatrienes.

1. Preparation of $\Delta^{8,12,14}$-Cholestatriene.

A solution of 1 g. of $\Delta^{8,14}$-cholestanene-$\gamma$-ol (41) in 50 cc. of alcohol containing 5 drops of concentrated hydrochloric acid was refluxed thirty minutes, cooled rapidly, filtered, taken up in 50 cc. of petroleum ether
(Shelly A) and passed through a 15 x 60 cm. column of activated alumina.
The alumina column was washed with 75 cc. of petroleum ether and the com-
bined filtrates were concentrated in vaccuo. The residue was crystallized
from acetone to yield 0.8 gm. of needles, m.p. 72-80°, (c) 0.21 -5.6° (c, 2.62
in carbon tetrachloride).

Anal. Calcd. for C_{29}H_{40}: C, 86.45; H, 11.55. Found: C, 86.21, 86.31;
H, 11.52, 11.55.

2. Preparation of \( \Delta^{3,6,9} \)-Cholestatriene.

a. Preparation of \( \Delta^{3,6,9} \)-Cholestadiene-7-ol. A solution of 3 gm. of
\( \Delta^{3,6} \)-cholestadiene-7-one in 150 cc. of anhydrous isopropyl alcohol contain-
ing 12 gm. of aluminum isopropanoxide was refluxed for four hours, and then
slowly distilled for an additional six hours. The solution was cooled, and
poured into 300 cc. of cold 3 per cent potassium hydroxide. After standing
for an hour, the mixture was extracted with ether. The ether extract was
washed, dried over sodium sulfate, and concentrated in vaccuo. The residue
was crystallized from acetone to yield 5.2 gm. of tablets which after
several recrystallizations from petroleum ether (Shelly B) melted at 92-93°.

Anal. Calcd. for C_{29}H_{38}O: C, 85.30; H, 11.92. Found: C, 84.66, 84.32;
H, 11.71, 11.62.

b. Dehydration of \( \Delta^{3,6} \)-Cholestadiene-7-ol. A solution of 1 gm. of
\( \Delta^{3,6} \)-cholestadiene-7-ol in 60 cc. of alcohol containing 6 drops of concen-
trated hydrochloric acid was refluxed thirty minutes under nitrogen. The
solution was cooled rapidly in a freezing bath and filtered; the crude
crystals were dissolved in 50 cc. of petroleum ether (Shelly A) and passed
through an 15 x 60 cm. column of activated alumina. The column was washed
with 50 cc. of the solvent and the combined filtrates were concentrated in
vacuo. The residue was crystallized from acetone to yield 0.45 gm. of needles,
m.p. 75-76°, [α]D^12 +17.2° (c, 1.50 in carbon tetrachloride).

Anal. Calcd. for C_{24}H_{30}O: C, 88.45; H, 11.55. Found: C, 88.27; H, 11.78;
F, 11.65; 11.76.
IV. DISCUSSION

Prior to the preparation of cholestane derivatives possessing mono- or di-unsaturation in rings C and D, the structure of "7-dehydrocholestanone isomer" was investigated since "7-dehydrocholestanone isomer" should possess two double bonds in conjugation either in the 2,6-positions or in rings C and D. It was found that no structure other than \( \Delta^{2,6} \)-cholestenone could account for the reactions of "7-dehydrocholestanone isomer". Thus, "7-dehydrocholestanone isomer" was converted to \( \Delta^{2,5} \)-cholestadiene by the action of hydrogen chloride in chloroform; catalytic hydrogenation of the "7-dehydrocholestanone isomer" yielded a mixture of cholestane and campestane, and \( \Delta^{2} \)-cholestane was obtained by the reduction of the chromic acid oxidation product of the isomer.

Since "7-dehydrocholestanone isomer" was thus found to have the structure of \( \Delta^{2,6} \)-cholestadiene, the constitution of the 5,6-dibromocholestane-quinoline product and the reduction product of \( \Delta^{2,6} \)-cholestadiene-3-one required investigation. The 5,6-dibromocholestane-quinoline product was found to be an inseparable mixture containing \( \Delta^{2} \)-cholestane since \( \Delta^{2} \)-cholestanone was isolated as its dibromide by bromination. The reduction product of \( \Delta^{2,6} \)-cholestadiene-3-one was found to contain \( \Delta^{2,5} \)-cholestadiene since the same cholestenolone was isolated from its chronic acid oxidation product as was obtained from \( \Delta^{2,6} \)-cholestadiene.

Two cholestatrenes were prepared from convenient intermediate compounds used in the preparation of cholestanes and cholestanones. Thus \( \Delta^{2,4,6} \)-cholestatrine was obtained by the dehydration of \( \Delta^{2,6} \)-cholestatene-3-one which was prepared by the reduction of \( \Delta^{2,6} \)-cholestanone-7-one. Likewise, \( \Delta^{3,5,7} \)-cholestatrine was obtained by dehydration of
$\Delta^8,\text{cholestadiene-7-ol}$ which was prepared by the reduction of $\Delta^7,\text{cholestadiene-7-one}$. $\Delta^2,\text{cholestadiene-7-one}$ is an intermediate compound in the preparation of cholestan-7-ol, which comprises the conversion of cholesterol to cholesteryl acetate to 7-ketocholesteryl acetate to $\Delta^3,\text{cholestadiene-7-one}$ to cholestan-7-one to cholestan-7-ol.

The preparation of $\Delta^5,\Delta^8(14)$- and $\Delta^{14}$-cholestenes was conducted in order to make a comparison of certain properties of various non-unsaturated derivatives of cholestan, as formulated in Figure 2.

$\Delta^8$-Cholesten was prepared by the dehydration of cholestan-7-ol with anhydrous copper sulfate in xylene in the presence of propionic acid. This method of preparation of a $\Delta^8$-unsaturated derivative is different than the method used in the preparation of previously known $\Delta^5$-unsaturated steroid derivatives. Thus $\Delta^5$-cholestenol and $\Delta^5$-coaprostanol were obtained by the sodium-propyl alcohol reduction of $\Delta^6,\Delta^8$-cholestadiene-7-ol and $\Delta^{14}$-coaprostadiene-7-ol, respectively.

Propionic acid was added to the reaction mixture in the preparation of $\Delta^8$-cholesten in order to obtain consistent results since the use of acid-free xylene was found to result in the dehydration of cholestan-7-ol to a mixture of $\Delta^8$- and $\Delta^8(14)$-cholestenes which were separated with difficulty by fractional crystallization.

The dehydration of cholestan-7-ol with activated alumina under mild conditions for a partial dehydration yielded $\Delta^8$-cholesten, whereas dehydration under more strenuous conditions yielded a mixture of about equal amounts of $\Delta^8$- and $\Delta^8(14)$-cholestenes. Similar to this dehydration, the dehydration of cholic acid does not yield the corresponding $\Delta^7$-unsaturated derivative but did, however, yield a mixture of the $\Delta^8(14)$- and $\Delta^{14}$-unsaturated derivatives.
Figure 2
Although $\Delta^{\text{B(14)}}$-cholestone was isolated from certain dehydration products of cholestan-7-ol, it was most conveniently prepared by shaking $\Delta^8$-cholestone in ethyl acetate with palladium catalyst and hydrogen. The mixture of $\Delta^8$- and $\Delta^{\text{B(14)}}$-cholestones, as obtainable by dehydration of cholestan-7-ol, was found to be conveniently converted to $\Delta^{\text{B(14)}}$-cholestone by this procedure. $\Delta^{14}$-Cholestone was prepared by the treatment of either $\Delta^8$- or $\Delta^{\text{B(14)}}$-cholestone with hydrogen chloride in chloroform solution.

The structure of $\Delta^8$-cholestone was determined by chronic acid oxidation. Only one monoketone was indicated to be present in the ketone fraction of the chronic acid oxidation product and this monoketone was found to be a cholestenone. The cholestenone was reduced with sodium and amyl alcohol and the reduction product on chronic acid oxidation yielded cholestan-7-one. The cholestenone was indicated, therefore, to be $\Delta^8$-cholesten-7-one.

The structures of $\Delta^{\text{B(14)}}$- and $\Delta^{14}$-cholestones were assigned by a comparison of their methods of preparation and their properties with analogous $\Delta^{\text{B(14)}}$- and $\Delta^{14}$-unsaturated derivatives. The structure of $\Delta^{\text{B(14)}}$-cholestone was assigned since $\Delta^{\text{B(14)}}$-cholestone was formed from $\Delta^8$-cholestone by shaking with palladium catalyst and hydrogen and was resistant to normal hydrogenation. This is in analogy with the formation of $\alpha$-cholestenol, $\alpha$-ergostenol and $\alpha$-coprostanol and with the resistance to normal hydrogenation of $\alpha$-spocholic acid and $\alpha_1$-alcohocistosterol acetate.

The structure of $\Delta^{14}$-cholestone was assigned since various $\Delta^{14}$-unsaturated steroid derivatives are formed by the action of hydrogen chloride in chloroform on the corresponding $\Delta^{\text{B(14)}}$-unsaturated derivatives and since normal hydrogenation of $\Delta^{14}$-cholestone yielded cholestone in analogy with
the normal hydrogenation of various \( \Delta^2 \)-unsaturated steroid derivatives to yield the corresponding saturated derivatives. The preparation of \( \Delta^{14} \)-cholestanone is analogous with the formation of \( \Delta^5 \)-cholestanol, \( \Delta^5 \)-ergostenol and \( \Delta^5 \)-desoxylessinates which are reduced normally and yield cholestanol, ergostenol and altostanol, respectively.

Various properties of the \( \Delta^6 \), \( \Delta^{6(14)} \) and \( \Delta^{14} \)-cholestanones were compared. It was found that the differences in specific rotation (Table IV) between the three cholestanones and cholestanone are in agreement with the average differences in specific rotation between various \( \Delta^6 \), \( \Delta^{6(14)} \) and \( \Delta^{14} \)-unsaturated steroid derivatives. The specific rotations of \( \Delta^6 \) and \( \Delta^{6(14)} \)-cholestanones agree with the generalization that the \( \Delta^{6(14)} \)-unsaturated steroid derivatives are less dextrorotatory than the corresponding saturated derivatives, whereas \( \Delta^{14} \)-unsaturated derivatives are more dextrorotatory.

Table IV.

Specific Rotations of Various Mono-unsaturated Steroid Derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \Delta^7 )</th>
<th>( \Delta^8 )</th>
<th>( \Delta^{6(14)} )</th>
<th>( \Delta^{14} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholestan-3-ol</td>
<td>+29.7</td>
<td>+12.2</td>
<td>+20.4</td>
<td>+21.0</td>
</tr>
<tr>
<td>Ergostenone</td>
<td>+11.0</td>
<td>+16.1</td>
<td>+18.4</td>
<td>+23.0</td>
</tr>
<tr>
<td>Ergostenan-3-ol</td>
<td>+15.6</td>
<td>-6.0</td>
<td>+15.1</td>
<td>+20.5</td>
</tr>
<tr>
<td>Sitosten-3-ol</td>
<td>+21.3</td>
<td>+11.2</td>
<td>+18.8</td>
<td>+22.4</td>
</tr>
<tr>
<td>Controstern-3-ol</td>
<td>+25.0</td>
<td>+15.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average difference(^a)</td>
<td>-23.6</td>
<td>-15.35</td>
<td>-21.15</td>
<td>+3.65</td>
</tr>
<tr>
<td>Cholestanone</td>
<td>+20.5</td>
<td>+11.2</td>
<td>+18.8</td>
<td>+22.4</td>
</tr>
<tr>
<td>Difference(^b)</td>
<td>-11.0</td>
<td>-1.0</td>
<td>+1.6</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Average difference in specific rotation between corresponding unsaturated and saturated derivatives.

\(^b\)Difference in specific rotation between the cholestanone and cholestanone.

A normal catalytic hydrogenation of \( \Delta^{14} \)-cholestanone was found to yield cholestanone, whereas \( \Delta^6 \)-cholestanone is rearranged to \( \Delta^{6(14)} \)-cholestanone and
\( \Delta^6(14) \)-cholestene remains unchanged. \( \Delta^8 \), \( \Delta^6(14) \) and \( \Delta^{8,14} \)-cholestadienes were found by titration to consume about three molar equivalents of bromine and about two molar equivalents of perbenzoic acid.

The preparation of \( \Delta^7,8(11) \), \( \Delta^7,14 \) and \( \Delta^{8,14} \)-cholestadienes was accomplished by introducing additional unsaturation into the \( \Delta^8 \) and \( \Delta^6(14) \)-cholestanes. Treatment of \( \Delta^8 \)-cholestane with bromine yielded \( \Delta^7,8(11) \)-cholestadiene, the structure of which was assigned from its method of preparation, its absorption spectra maximum at 298 \( \text{nm} \) and its failure to yield a maleic anhydride addition product. Treatment of \( \Delta^8 \)-cholestane with perbenzoic acid yielded \( \Delta^7,14 \)-cholestadiene, the structure of which was assigned from its strong levorotation, its absorption spectra maxima at 285 and 250 \( \text{nm} \) and its formation of a maleic anhydride addition product.

\( \Delta^{8,14} \)-Cholestadiene was prepared from \( \Delta^6(14) \)-cholestane by the action of perbenzoic acid, selenium dioxide, bromine, and chronic acid. The structure of \( \Delta^{8,14} \)-cholestadiene was assigned from its methods of preparation, its absorption spectra maximum at 285 \( \text{nm} \) and its failure to yield a maleic anhydride addition product.

The \( \Delta^7,8(11) \), \( \Delta^7,14 \) and \( \Delta^{8,14} \)-cholestadienes were found to consume about three molar equivalents of bromine in chloroform solution and slightly less than two molar equivalents of bromine in methanol solution.
V. SUMMARY

"1a-Dihydrocholestone isomer" was found to have the structure of \( \Delta^1\)-cholesterol. Accordingly, \( \Delta^1\)-cholesterol has a melting point of 90-91 °C and a specific rotation of +44.27 °.

In a study of various cholestane derivatives possessing unsaturation in or adjacent to rings C and D, \( \Delta^5\), \( \Delta^6\), \( \Delta^7\), \( \Delta^7\)-cholestane and \( \Delta^5\)-cholesterol and \( \Delta^7\)-cholesterol were prepared.

Various properties of the \( \Delta^5\), \( \Delta^6\), \( \Delta^7\)-cholestane were compared. The three cholestanes were found on titration to consume about three molar equivalents of resorcinol in chloroform solution. The consumption of more than the theoretical molar equivalent of resorcinol is accounted for by the spontaneous cleavage of hydrogen bromide to result in additional unsaturation. The three cholestanes were found also on titration to consume about two molar equivalents of paraformaldehyde. Partial catalytic hydrogenation of \( \Delta^5\)-cholesterol was found to yield cholestane, whereas \( \Delta^7\)-cholesterol is converted to \( \Delta^7\)-cholesterol and \( \Delta^8\)-cholesterol remains unchanged. \( \Delta^8\)-cholesterol was found to yield \( \Delta^5\)-cholesterol when dehydrogenated with bromine, paraformaldehyde, selenium dioxide or chromic acid. \( \Delta^5\)-Cholestane was found to be converted to \( \Delta^7\)-cholesterol and \( \Delta^7\)-cholesterol by the action of bromine and to \( \Delta^1\)-cholesterol by the action of paraformaldehyde.

From intermediate compounds in connection with the preparation of cholestanes and cholesteroles, \( \Delta^7\)-cholesterol and \( \Delta^1\)-cholesterol were prepared.
VI. LITERATURE CITED


42. Petrov, V. A. Private communication to Dr. J. E. Nek. 1933. Ames.


I was born on September 30, 1911 at Greenwood, South Carolina. My elementary and high school training was obtained in the public schools of Greenwood. I entered Clemson Agricultural and Mechanical College in 1929 and graduated in 1933 with the degree of Bachelor of Science in Textile Chemistry. My graduate work began at Louisiana State University. I remained there for two years as a fellow in chemistry; the degree of Master of Science was conferred upon me in 1935. I entered the Graduate School of Iowa State College in 1937 and have held a Graduate Research Assistantship in the Animal Chemistry and Nutrition Subsection from 1937 to the present date.