This $\alpha,\beta$-diaminoketone was not soluble in 1-2 *N* hydrochloric acid but dissolved only in more concd. acids. Heating the acid solution in a water-bath gave benzaldehyde (60% yield) but no benzyl phenyl diketone was noted.

**$\alpha$-N-Piperidino-$\beta$-N-tetrahydroquinolinobenzylacetophenone, V.**—$\alpha$-Bromo-$\alpha$-N-piperidinobenzylacetophenone (10.0 g.) prepared according to the method of Dufraisse[2] was suspended in 18 ml. of ethanol and mixed with 7.15 g. (two equiv.) of tetrahydroquinoline. A very slow reaction set in and six hours had passed before all of the bromide dissolved. After standing for two days the mixture was cooled to give 8.2 g. of a yellow crystalline precipitate, m. p. 164–166°. Recrystallization from benzene and petroleum ether gave 7.2 g. of pale yellow needles, m. p. 166–167°.

*Anal.* Calcd. for $C_{29}H_{32}N_2O$: C, 82.03; H, 7.60. Found: C, 81.84; H, 7.81.

This diaminoketone was only slowly soluble in warm dilute mineral acids.

**Hydrolysis of V.**—The diamino ketone V (3.0 g.) was heated on the steam-bath with 30 ml. of 15% sulfuric acid for one and one-half hours. The precipitated oil (0.38 g.) was shown to be only benzaldehyde.

On neutralization of the residual acid solution an oily precipitate was obtained. This was treated with benzene sulfonyl chloride in alkaline solution to remove the tetrahydroquinoline as the benzene sulfonamide. The oily product from this reaction was dissolved in ether and the unchanged amines present extracted with dilute hydrochloric acid. This acid solution on neutralization gave an oily precipitate which was taken up in ether. On passing dry hydrogen chloride into this latter solution, a white precipitate (0.50 g.) was obtained which was shown to be identical with the hydrochloride of $\omega$-piperidinoacetophenone.[1]

**$\alpha$-N-Morpholino-$\beta$-N-tetrahydroquinolinobenzylacetophenone, VI.**—$\alpha$-Bromo-$\alpha$-N-morpholinobenzylacetophenone[3] (3.5 g.) was mixed with 2.5 g. (2 equiv.) of tetrahydroquinoline in 8 ml. of ethanol. After standing for twenty-four hours only a small amount of the bromide had reacted. The mixture was heated at 50° for two hours and then cooled in the ice chest for twenty hours. A pale green precipitate, 2.18 g., was filtered off, m. p. 151–153°. This product on recrystallization from benzene and petroleum ether gave pale, yellow-green needles (1.5 g.), m. p. 153–154°.

*Anal.* Calcd. for $C_{28}H_{30}N_2O_2$: C, 78.84; H, 7.09. Found: C, 78.61; H, 7.33.

This product was not soluble in cold dilute mineral acids but dissolved slowly in warm solutions.

## Summary

1. Pyrrolidine has been found to resemble piperidine in its reactions with $\alpha$-bromobenzalacetophenone and benzalacetophenone dibromide. Tetrahydroquinoline did not react with these bromides.

2. A new $\alpha$-bromo-$\alpha$-aminoketone, one new $\alpha$-amino-$\alpha,\beta$-unsaturated ketone, and four new $\alpha,\beta$-diaminoketones have been prepared.

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[CONTRIBUTION FROM THE ANIMAL CHEMISTRY AND NUTRITION SUBSECTION OF IOWA STATE COLLEGE]

# The Preparation of $\Delta^8$-, $\Delta^{8(14)}$- and $\Delta^{14}$-Cholestenes[1]

BY J. C. ECK AND E. W. HOLLINGSWORTH[2]

The preparation of $\Delta^8$-, $\Delta^{8(14)}$- and $\Delta^{14}$-cholestenes was conducted in order to make a comparison of certain properties of various mono-unsaturated derivatives of cholestane. These cholestenes are desirable as convenient cholestane derivatives for investigations of rings C and D. The double bonds are located in ring A or B in the hitherto known cholestenes which are $\Delta^2$-cholestene (neocholestene), $\Delta^4$-cholestene (pseudo-cholestene or coprostene) and $\Delta^5$-cholestene (cholestene). The methods of preparation and the specific rotations of the $\Delta^8$-, $\Delta^{8(14)}$- and $\Delta^{14}$-cholestenes were compared also with those of the analogous $\Delta^8$-, $\Delta^{8(14)}$- and $\Delta^{14}$-unsaturated steroid derivatives.

$\Delta^8$-Cholestene 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 from the method used in the preparation of previously known $\Delta^8$-unsaturated sterol derivatives. Thus, the $\Delta^8$-unsaturated derivatives of cholestan-3-ol[3] and coprostan-3-ol[4] were obtained by the sodium–propyl alcohol reduction of the corresponding $\Delta^{6,8}$-unsaturated derivatives in a manner similar to the preparation of the $\Delta^7$-unsaturated derivatives of cholestan-3-ol[5] and ergostan-3-ol[6] by the sodium–ethyl alcohol reduction of the corresponding $\Delta^{5,7}$-unsaturated derivatives.

(1) Journal Paper No. J897 of the Iowa Agricultural Experiment Station, Project No. 506.

(2) Submitted from unpublished research conducted by E. W. Hollingsworth in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(3) Windaus, Linsert and Eckhardt, *Ann.*, **534**, 22 (1938).

(4) Windaus and Zühlsdorff, *ibid.*, **536**, 204 (1938).

(5) Schenck, Buchholz and Wiese, *Ber.*, **69B**, 2698 (1936).

(6) Windaus and Langer, *Ann.*, **508**, 105 (1933).

Propionic acid was added to the reaction mixture in the preparation of $\Delta^8$-cholestene in order to obtain consistent results since the use of acid-free xylene resulted 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 presence of a small amount of propionic acid, however, with either technical or reagent grade xylene in the copper sulfate dehydration of cholestan-7-ol consistently yielded a dehydration product from which $\Delta^8$-cholestene was conveniently isolated. The dehydration of cholestan-7-ol with activated alumina under mild conditions for a partial dehydration yielded $\Delta^8$-cholestene, whereas dehydration under more strenuous conditions yielded a mixture of about equal amounts of $\Delta^8$- and $\Delta^{8(14)}$-cholestenes.

Although $\Delta^{8(14)}$-cholestene was isolated from certain dehydration products of cholestan-7-ol, it was most conveniently prepared by shaking $\Delta^8$-cholestene in ethyl acetate solution with palladium catalyst and hydrogen. The mixture of $\Delta^8$- and $\Delta^{8(14)}$-cholestenes, as obtainable by dehydration of cholestan-7-ol, was found to be conveniently converted to $\Delta^{8(14)}$-cholestene by this procedure. $\Delta^{14}$-Cholestene was prepared by the treatment of either $\Delta^8$- or $\Delta^{8(14)}$-cholestene with hydrogen chloride in chloroform solution. In addition to the $\Delta^{14}$-cholestene, a cholestanol of unknown structure was isolated from the reaction product. This cholestanol is considered to have resulted from a side reaction in a manner similar to the formation of the molecular compound of *allo*- and *epi-allo*-cholesterol in the conversion of $\Delta^{4,6}$-cholestadiene to $\Delta^{3,5}$-cholestadiene by the action of hydrogen chloride in chloroform solution.[7]

The structure of $\Delta^8$-cholestene was determined by chromic acid oxidation. Only one mono-ketone was indicated to be present in the ketone fraction of the chromic acid oxidation product of $\Delta^8$-cholestene and the mono-ketone was found to be a cholestenone. The cholestenone was reduced with sodium and amyl alcohol and the reduction product on chromic acid oxidation yielded cholestan-7-one. The cholestenone was indicated, accordingly, to be $\Delta^8$-cholesten-7-one. The method of preparation of $\Delta^8$-cholestene could indicate a $\Delta^7$-unsaturated structure but the corresponding cholestanone from a $\Delta^7$-unsaturated derivative would be cholestan-6-one.

The structures of $\Delta^{8(14)}$- and $\Delta^{14}$-cholestenes were determined by a comparison of their methods of preparation and of their properties with analogous $\Delta^{8(14)}$- and $\Delta^{14}$-unsaturated sterol derivatives. The structure of $\Delta^{8(14)}$-cholestene was assigned since $\Delta^{8(14)}$-cholestene was formed from $\Delta^8$-cholestene by shaking with palladium catalyst and hydrogen and is resistant to normal hydrogenation. This is in analogy with the formation of various $\Delta^{8(14)}$-unsaturated sterol derivatives such as $\Delta^{8(14)}$-cholesten-3-ol ($\alpha$-cholestenol) from $\Delta^7$-cholesten-3-ol ($\gamma$-cholestenol)[5] and from $\Delta^8$-cholesten-3-ol ($\delta$-cholestenol,)[8] $\Delta^{8(14)}$-ergosten-3-ol ($\alpha$-ergostenol) from $\Delta^7$-ergosten-3-ol ($\gamma$-ergostenol)[8] and $\Delta^{8(14)}$-coprosten-3-ol ($\alpha$-coprostenol) from $\Delta^8$-coprosten-3-ol ($\delta$-coprostenol).[4] This is also in agreement with the resistance to normal hydrogenation of $\Delta^{8(14)}$-$\alpha_1$-sitosten-3-ol acetate ($\alpha_1$-dihydrositosteryl acetate).[9] It should be pointed out in this connection, however, that $\Delta^{8(14)}$-unsaturated steroid derivatives or those unsaturated steroid derivatives which yield $\Delta^{8(14)}$-unsaturated derivatives under the conditions for normal hydrogenation, can be hydrogenated in the presence of hydrochloric acid at an elevated temperature (65° to 80°) to yield the corresponding saturated derivatives.[3,4,9,10]

The structure of $\Delta^{14}$-cholestene was assigned since various $\Delta^{14}$-unsaturated sterol derivatives are formed by the action of hydrogen chloride in chloroform solution on the corresponding $\Delta^{8(14)}$-unsaturated derivatives and since normal hydrogenation of $\Delta^{14}$-cholestene yielded cholestane in analogy with the normal hydrogenation of various $\Delta^{14}$-unsaturated sterol derivatives to yield the corresponding saturated derivatives. Thus, $\Delta^{8(14)}$-cholesten-3-ol is rearranged to $\Delta^{14}$-cholesten-3-ol ($\beta$-cholestenol),[5] the acetate of which yields cholesten-3-ol acetate on normal hydrogenation[5]; $\Delta^7$-ergosten-3-ol (benzoate)[6] and $\Delta^{8(14)}$-ergosten-3-ol (acetate)[11] are rearranged to $\Delta^{14}$-ergosten-3-ol ($\beta$-ergostenol) which yields ergostan-3-ol on normal hydrogenation[12] and $\Delta^{8(14)}$-$\alpha_1$-sitosten-3-ol acetate is rearranged to $\Delta^{14}$-$\alpha_1$-sitosten-3-ol acetate ($\alpha_1$-isodihydrositosteryl acetate)[9] which yields $\alpha_1$-sitostan-3-ol acetate on normal hydrogenation.[9]

The specific rotations of $\Delta^8$-, $\Delta^{8(14)}$- and $\Delta^{14}$-cholestenes support the structures assigned. It was found that the differences in specific rotation

(7) Eck and Hollingsworth, THIS JOURNAL, **63**, 2320 (1941).

(8) Reichel, *Z. physiol. Chem.*, **226**, 146 (1934).

(9) Bernstein and Wallis, THIS JOURNAL, **61**, 2308 (1939).

(10) Dithmar and Achtermann, *Z. physiol. Chem.*, **205**, 55 (1932).

(11) Reindel, Walter and Rauch, *Ann.*, **452**, 34 (1927).

(12) Heilbron and Wilkinson, *J. Chem. Soc.*, 1708 (1932).

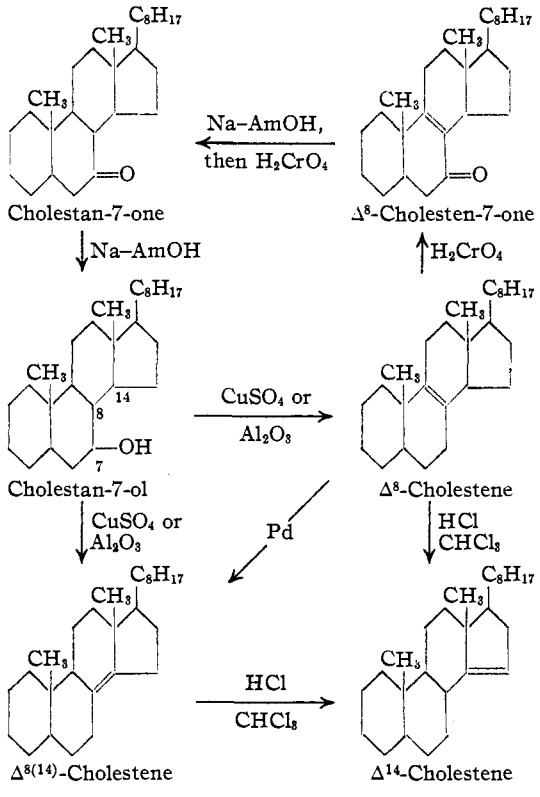


TABLE I

SPECIFIC ROTATIONS OF VARIOUS MONO-UNSATURATED STEROID DERIVATIVES

| | Sat'd. | $\Delta^7$- | $\Delta^8$- | $\Delta^{8(14)}$- | $\Delta^{14}$- |
|---|---|---|---|---|---|
| Cholestan-3-ol | +29.7 | 0.0 | +12.2 | +20.4 | +34.0 |
| Coprostan-3-ol | +28.0 | | +15.0 | | |
| Ergostane | +19.9 | | | +11.0 | +21.3 |
| Ergostan-3-ol | +15.5 | 0.0 | | +13.1 | +20.3 |
| Ergostan-3-one | +34.9 | | | +38.8 | +36.8 |
| $\alpha_1$-Sitostan-3-ol | +27.0 | | | +10.9 | +31.0 |
| Desoxycholic acid | +57.1 | | | | +60.1 |
| Av. difference[a] | | −22.6 | −15.25 | −6.56 | +3.23 |
| Cholestane | +24.8 | | +11.2 | +21.2 | +26.6 |
| Difference[b] | | | −13.6 | −3.6 | +1.8 |

[a] Average difference in specific rotation between corresponding unsaturated and saturated derivatives. [b] Difference in specific rotation between the cholestene and cholestane. Various solvents were used in the determination of the specific rotations listed.

(Table I) between the three cholestenes and cholestane are in agreement with the average differences in specific rotation between various $\Delta^8$-, $\Delta^{8(14)}$- and $\Delta^{14}$-unsaturated steroid derivatives and the corresponding saturated derivatives. It was also noted that the difference in specific rotation between $\Delta^8$-cholestene and cholestane is in better agreement with the average difference in specific rotation between the $\Delta^8$- than the $\Delta^7$-unsaturated steroid derivatives and the corresponding saturated derivatives. $\gamma$-Ergostenol is considered[13] to have the structure of $\Delta^7$-ergosten-3-ol although the difference (15.5°) in specific rotation between $\gamma$-ergostenol and ergostane is in better agreement with the average difference (15.25°) in specific rotation between the $\Delta^8$-unsaturated steroid derivatives and the corresponding saturated derivatives than with the difference (29.7°) in specific rotation between $\Delta^7$-cholesten-3-ol and cholestan-3-ol. The specific rotations of $\Delta^{8(14)}$- and $\Delta^{14}$-cholestenes agree with the generalization that the $\Delta^{8(14)}$-unsaturated steroid derivatives are less dextrorotatory than the corresponding saturated derivatives, whereas $\Delta^{14}$-unsaturated derivatives are more dextrorotatory. Dihydroxycholenic acid ($\Delta^{14}$-3,12-dihydroxycholenic acid)[14] was the only mono-unsaturated derivative of desoxycholic acid included in Table I since the structures of the other mono-unsaturated dehydration derivatives of cholic acid have not been established.

The properties of the various cholestenes vary considerably. Normal catalytic hydrogenation of $\Delta^2$-, $\Delta^5$- and $\Delta^{14}$-cholestenes yields cholestane and of $\Delta^4$-cholestene yields coprostane, whereas $\Delta^8$-cholestene is rearranged to $\Delta^{8(14)}$-cholestene and $\Delta^{8(14)}$-cholestene remains unchanged. The action of bromine on the $\Delta^8$-, $\Delta^{8(14)}$- and $\Delta^{14}$-cholestenes was found to differ from the action of bromine on the $\Delta^2$-, $\Delta^4$- and $\Delta^5$-cholestenes. Although $\Delta^2$-, $\Delta^4$- and $\Delta^5$-cholestenes add one molar equivalent of bromine with the formation of dibromides, the $\Delta^8$-, $\Delta^{8(14)}$- and $\Delta^{14}$-cholestenes were found by titration to consume about three molar equivalents of bromine in chloroform solution at 0° and bromine derivatives could not be isolated. The consumption of more than the theoretical molar equivalent of bromine by the $\Delta^8$-, $\Delta^{8(14)}$- and $\Delta^{14}$-cholestenes is accounted for by the spontaneous cleavage of hydrogen bromide to result in additional unsaturation. The solvent used was found to be of importance in the bromine titrations since the molar equivalents of bromine consumed were found to vary considerably with the conditions employed. The action of perbenzoic acid on the $\Delta^8$-, $\Delta^{8(14)}$- and $\Delta^{14}$-cholestenes was found also to differ from the action of perbenzoic acid on the $\Delta^2$-, $\Delta^4$- and $\Delta^5$-cholestenes. Although the $\Delta^2$-, $\Delta^4$- and $\Delta^5$-cholestenes are converted to oxides by the consumption of a molar equivalent of perbenzoic acid, the $\Delta^8$-, $\Delta^{8(14)}$- and $\Delta^{14}$-cholestenes

(13) Laucht, *Z. physiol. Chem.*, **237**, 236 (1935).
(14) Callow, *J. Chem. Soc.*, 462 (1936).

were found by titration to consume about two molar equivalents of perbenzoic acid.

## Experimental

**Preparation of Cholestan-7-ol.**—Cholestan-7-ol was most conveniently prepared by the sodium–amyl alcohol reduction of cholestan-7-one[15] although it was also prepared by the aluminum isopropoxide reduction of cholestan-7-one and by the catalytic hydrogenation of $\Delta^5$-cholesten-7-ol. Both the crude (m. p. 106–110°) and the purified (m. p. 118–119°) samples of cholestan-7-ol yielded the same dehydration product.

**Dehydration of Cholestan-7-ol.**—A mixture of 8 g. of cholestan-7-ol, 8 g. of finely powdered anhydrous copper sulfate, 40 cc. of either technical or reagent xylene and 0.2 cc. of propionic acid was refluxed for five hours in a 200-cc. Erlenmeyer flask provided with an air condenser. To the cooled reaction product was added 60 cc. of petroleum ether (b. p. 60–70°) and the decanted solution was passed through a 15 × 305 mm. column of activated alumina (preheated 50 to 200 mesh Alorco). The alumina column was washed with 200 cc. of petroleum ether and the combined filtrates were concentrated *in vacuo*. The residue was dissolved in 100 cc. of hot acetone and hot methanol was carefully added to the point of turbidity whereupon crystallization occurred on cooling to yield 3.6 g. of $\Delta^8$-cholestene in the form of plates, m. p. 70–77°. Repeated recrystallization from acetone–methanol yielded $\Delta^8$-cholestene, m. p. 85–86°, $(\alpha)^{18}D$ +11.2° (*c*, 3.54 in carbon tetrachloride), which showed no noticeable absorption in the region of 220 mμ to 280 mμ.

*Anal.* Calcd. for $C_{27}H_{46}$: C, 87.47; H, 12.53. Found: C, 87.35, 87.48; H, 12.40, 12.47.

The alumina column was eluted with 400 cc. of ether and the ether eluate was concentrated *in vacuo*. The residue was dissolved in hot acetone and water was carefully added with stirring to turbidity. The material was filtered when cold to yield 3.1 g. of unchanged cholestan-7-ol, m. p. 116–118°.

A mixture of 5.8 g. of cholestan-7-ol, 5.8 g. of anhydrous copper sulfate and 30 cc. of reagent grade xylene in a 125-cc. flask was refluxed for four hours. The column filtrate fraction of the reaction product weighed 4.9 g. which indicated that most of the cholestan-7-ol had been dehydrated. By a fractional crystallization from acetone, $\Delta^8$-cholestene was separated from $\Delta^{8(14)}$-cholestene. On repeated recrystallization from acetone, the $\Delta^{8(14)}$-cholestene was obtained as needles, m. p. 53–54°, $[\alpha]^{23}D$ +21.2° (*c*, 3.12 in carbon tetrachloride).

*Anal.* Calcd. for $C_{27}H_{46}$: C, 87.47; H, 12.53. Found: C, 87.59, 87.38; H, 12.46, 12.58.

A mixture of 0.5 g. of cholestan-7-ol and 0.5 g. of activated alumina (200 mesh) was immersed in a Wood's metal bath preheated to 160°. The temperature of the bath was raised during ten minutes to 260–270° where it was maintained for an additional fifteen minutes. The column filtrate fraction of the reaction product yielded 35 mg. of $\Delta^8$-cholestene. A duplicate reaction mixture was heated under nitrogen at 290–295° for forty-five minutes and the column filtrate fraction (0.22 g.) of the reaction product yielded the $\Delta^8$- and $\Delta^{8(14)}$-cholestenes on fractional crystallization.

**Conversion of $\Delta^8$-Cholestene to $\Delta^{8(14)}$-Cholestene.**—A solution of 3 g. of $\Delta^8$-cholestene dissolved in 75 cc. of ethyl acetate was shaken with 0.2 g. of palladium catalyst in the presence of hydrogen for three hours. The palladium was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was dissolved in hot acetone and the solution was cooled. An equal volume of methanol was added and the product was cooled in a freezing bath and filtered to yield 2.7 g. of $\Delta^{8(14)}$-cholestene in the form of needles, m. p. 52–54°. Repeated recrystallization from methanol raised the melting point of the $\Delta^{8(14)}$-cholestene to 53–54°. A mixture of $\Delta^8$- and $\Delta^{8(14)}$-cholestenes, obtained by dehydration of cholestan-7-ol, also yielded $\Delta^{8(14)}$-cholestene by this procedure.

**Preparation of $\Delta^{14}$-Cholestene.**—Dry hydrogen chloride was passed through a solution of 0.5 g. of $\Delta^{8(14)}$-cholestene dissolved in 20 cc. of chloroform at 0° for two hours. The solvent was removed *in vacuo* and the residue was dissolved in 75 cc. of ether. The ether solution was shaken with 10% sodium hydroxide solution for ten minutes, washed with water and dried over anhydrous sodium sulfate. The ether solution was concentrated *in vacuo* and a solution of the residue dissolved in 25 cc. of petroleum ether (b. p. 30–40°) was passed through a 8 × 70 mm. column of activated alumina. The column was washed with 25 cc. of petroleum ether and the combined filtrates were concentrated *in vacuo*. The residue was dissolved in 25 cc. of hot acetone and 25 cc. of methanol was added to the cold acetone solution over a period of one hour. The material was filtered to yield 0.21 g. of $\Delta^{14}$-cholestene in the form of plates which after several recrystallizations melted at 73–74° and $[\alpha]^{21}D$ was +26.6° (*c*, 3.08 in carbon tetrachloride). $\Delta^{14}$-Cholestene (0.15 g.) was isolated also from the reaction product obtained from 0.5 g. of $\Delta^8$-cholestene by this procedure.

*Anal.* Calcd. for $C_{27}H_{46}$: C, 87.47; H, 12.53. Found: C, 87.36, 87.22; H, 12.55, 12.67.

The alumina column was eluted with 50 cc. of ether and the eluate was concentrated *in vacuo*. The residue was dissolved in 10 cc. of acetone and 5 cc. of methanol was added. After crystallization for several days, 30 mg. of halogen-free needles was removed by filtration. After several recrystallizations from acetone the compound melted at 119–120° and $(\alpha)^{22}D$ was +37.1° (*c*, 1.55 in carbon tetrachloride). This compound was indicated to be a cholestanol from its analysis and since it was recovered unchanged following treatment with alcoholic hydrochloric acid and did not add bromine.

*Anal.* Calcd. for $C_{27}H_{48}O$: C, 83.42; H, 12.46. Found: C, 83.63; H, 12.42.

**Catalytic Hydrogenation of $\Delta^{14}$-Cholestene.**—A mixture of 50 mg. of $\Delta^{14}$-cholestene, 50 cc. of ethyl acetate and 100 mg. of platinum oxide was shaken with hydrogen at room temperature for six hours. The reduction product in carbon tetrachloride solution was treated with sulfuric acid and acetic anhydride and upon crystallization from 30 cc. of absolute alcohol yielded 29 mg. of a compound in plates which melted at 79–80° and which gave no depression in mixed melting point with an authentic sample of cholestane.

**Conversion of $\Delta^8$-Cholestene to Cholestan-7-one.**—A mixture of 10 g. of $\Delta^8$-cholestene, 200 cc. of benzene, 80 cc.

(15) Heilbron, Shaw and Spring, *Rec. trav. chim.*, **57**, 529 (1938).

of acetic acid and a solution of 10 g. of chromic anhydride dissolved in 140 cc. of dilute sulfuric acid (1:3) was stirred at room temperature for six hours. 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, 5% sodium hydroxide solution and with water. The solution was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue dissolved in 100 cc. of petroleum ether, was passed through a 18 × 240 mm. column of activated alumina and 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 2.4 g. of unchanged $\Delta^8$-cholestene, m. p. 85–86°, which was indicated to be contaminated slightly with a diene by absorption spectra data.

The alumina column was eluted with 600 cc. of ether and the ether eluate was concentrated *in vacuo* to yield 4.2 g. of an oil. Slow crystallization of the oil from methanol yielded 1.4 g. of a compound which was repeatedly recrystallized from methanol, m. p. 86.5–87.5°, $[\alpha]^{21}D$ +3.8° (*c*, 2.15 in carbon tetrachloride). The $\alpha,\beta$-unsaturated ketone structure of a cholestenone was indicated for the compound by its analysis and by an absorption spectra maximum at 251 mμ.

*Anal.* Calcd. for $C_{27}H_{44}O$: C, 84.30; H, 11.53. Found: C, 84.08, 83.95; H, 11.60, 11.54.

The filtrates from the methanol crystallizations of the cholestenone contained the remaining 2.8 g. of the ketone fraction. These filtrates were concentrated *in vacuo* and the residue was indicated by its analysis to contain essentially only diketones. A diketone was isolated in the form of needles which melted at 74–75° and $[\alpha]^{24}D$ was −53.8° (*c*, 1.30 in carbon tetrachloride).

*Anal.* Calcd. for $C_{27}H_{44}O_2$: C, 80.93; H, 11.08. Found: C, 81.13; H, 10.32 for the diketone fraction. Found: C, 81.17, 81.25; H, 10.68, 10.77 for the diketone.

The cholestenone was reduced as follows. During a period of two hours, 2 g. of sodium was added to a boiling solution of 500 mg. of the cholestenone dissolved in 50 cc. of amyl alcohol. After cooling, 15 cc. of water was added and the water layer was discarded after occasional shaking during the period of one hour. The alcohol solution was diluted with 100 cc. of ether and this solution was washed with water and concentrated *in vacuo*. The residue was dissolved in hot methanol and water was carefully added to turbidity to yield 310 mg. of a semi-crystalline product. Chromic acid oxidation of this product yielded 190 mg. of a cholestanone which was repeatedly recrystallized from methanol, m. p. 115–116° (oxime, m. p. 134–135°) and which did not add bromine. An authentic sample of cholestan-7-one was prepared and its melting point (m. p. 108–109°) was found to agree with that reported.[16] Purification of this sample of cholestan-7-one by treatment with chromic acid, however, yielded cholestan-7-one, m. p. 113–114° (oxime, m. p. 134–135°). No depression in mixed melting point was obtained for the cholestanone with cholestan-7-one or for the oxime of the cholestanone with the oxime of cholestan-7-one.

**Bromine Titrations.**—A chloroform solution of 20 mg. of each of the cholestenes was titrated with a 0.1 *N* chloroform solution of bromine. The unconsumed bromine which remained after standing at 0° for five minutes was determined by the addition of potassium iodide followed by titration with standard sodium thiosulfate solution. $\Delta^5$-Cholestene, $\Delta^8$-cholestene, $\Delta^{8(14)}$-cholestene and $\Delta^{14}$-cholestene consumed 1.02, 3.00, 3.37 and 2.92 molar equivalents of bromine, respectively. On titration with bromine in methanol, the same cholestenes in methanol solution consumed 1.07, 1.71, 2.15 and 1.31 molar equivalents of bromine, respectively. On titration with bromine in chloroform solution, $\Delta^8$-cholestene consumed 1.70 molar equivalents of bromine when dissolved in ether and 2.49 molar equivalents of bromine when dissolved in acetic acid.

**Perbenzoic Acid Titrations.**—In a 50-cc. Erlenmeyer flask was placed 7 cc. of a chloroform solution of perbenzoic acid containing three times the theoretical amount of oxygen required for 100 mg. of cholestene, and 100 mg. of the cholestene. The samples together with blanks were kept at 0° for seven days and then the excess oxygen was titrated with standard sodium thiosulfate after the addition of potassium iodide. $\Delta^5$-Cholestene, $\Delta^8$-cholestene, $\Delta^{8(14)}$-cholestene and $\Delta^{14}$-cholestene absorbed 4.45, 9.10, 8.18 and 7.83 mg. of oxygen which corresponds to 1.03, 2.11, 1.89 and 1.81 atom equivalents, respectively.

## Summary

$\Delta^8$-, $\Delta^{8(14)}$- and $\Delta^{14}$-cholestenes were prepared and the structure of $\Delta^8$-cholestene was established by its conversion to cholestan-7-one. The normal hydrogenation and the consumption of bromine and of perbenzoic acid by cholestenes were compared. The specific rotations of $\Delta^8$-, $\Delta^{8(14)}$- and $\Delta^{14}$-cholestenes were found to be in agreement with the specific rotations of the analogous $\Delta^8$-, $\Delta^{8(14)}$- and $\Delta^{14}$-unsaturated steroid derivatives, respectively ($\delta$-, $\alpha$- and $\beta$-stenols, respectively).

AMES, IOWA RECEIVED JULY 3, 1941

(16) Windaus and Kircher, *Ber.*, **53**, 614 (1920).