alcohol, the ester hydrochloride was obtained in yield of 9.5 g. (40%); m. p. 177–178° (dec.).

Anal. Calcd. for $C_{24}H_{28}O_3N_3Cl$: N, 6.79. Found: N, 6.75.

Pharmacological.—Pharmacological data included in Tables II, IV and V were obtained at the Merck Institute for Therapeutic Research, and will be published elsewhere by Albert O. Seeler and Samuel Kuna. The method of testing is outlined in ref. 2a.

Summary

Several 3-alkylamino-1-propanols have been prepared by hydrogenating ketone-3-amino-1-propanol mixtures, or by hydrogenating the anhydro compounds formed by condensation of ketones with 3-amino-1-propanol. *p*-Nitro and *p*-aminobenzoate hydrochlorides have been synthesized from the 3-alkylamino-1-propanols by methods previously used for preparing similar alkylaminoethanol derivatives.

Several new *p*-aminobenzoate hydrochlorides of 2-dialkylaminoethanols and 1-dialkylamino-2-propanols have been prepared for comparison with similar monoalkylamino derivatives.

p-Dimethylaminobenzoate and *p*-diethylaminobenzoate hydrochlorides of representative 2-alkylaminoethanols, 1-alkylamino-2-propanols and 2-alkylamino-1-butanols have been synthesized.

These esters have been examined for local anesthetic activity.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

The Action of Platinum on Cholesterol in Acetic Acid Solution

BY HERBERT S. ANKER[1] AND KONRAD BLOCH

In the preparation of deuterio cholesterol in this Laboratory[2] by treatment of cholesterol with deuterium-containing acetic acid, heavy water and active platinum at elevated temperatures, it was observed that other compounds besides deuterio cholesterol were formed in the reaction. The amount of cholesterol recovered was dependent on the length of the treatment, the concentration of acetic acid and the amount of catalyst present. The conditions which favored the introduction of deuterium into the sterol molecule also favored its decomposition. Preliminary tests indicated that the products formed were of the nature of steroid ketones and hydrocarbons. As these products arise in the absence of any oxidizing or reducing agents they must have been formed by intermolecular hydrogen transfer.

In order to identify the products formed, cholesterol was subjected to the same conditions which had led to the formation of deuterio cholesterol, except that ordinary dilute acetic acid was used. Moreover, the products isolated from the experiment carried out in an isotopic medium were analyzed for deuterium in order to obtain some information on the mechanism of the reactions.

The free alcohol group of the cholesterol molecule appears to be necessary for the introduction of deuterium and for the occurrence of chemical changes since cholesteryl chloride and *i*-cholesteryl methyl ether remain unchanged on such treatment.[2] The influence of the double bond on the nature of the chemical changes and the exchange reaction was studied by analogous treatment of dihydrocholesterol.

Experimental

Action of Platinum and Dilute Acetic Acid on Cholesterol

Fifty grams of cholesterol purified via the dibromide[3] was shaken for one hundred hours at 127° in sealed flasks with 240 ml. of 50% acetic acid and platinum obtained by the reduction of 6 g. of platinum oxide. The reaction mixture was dissolved in ether, filtered, washed several times with 0.5% sodium bicarbonate solution, then with water and dried. The ether was then removed.

Acids.—In order to saponify the partially acetylated reaction products the residue was dissolved in 1.3 liters of 2% ethanolic potassium hydroxide. After three days at room temperature the reaction mixture was diluted with water and extracted with ether. The alkaline layer on acidification yielded 0.69 g. of acidic material. By titration in ethanol with sodium hydroxide an equivalent weight of 330 ± 10 was found. After esterification with diazomethane determination of the molecular weight by the micro method of Rast gave a value of 406 ± 40. No attempt was made further to characterize this small fraction.

Ketones.—The neutral fraction (42 g.), on treatment with *p*-hydrazinobenzoic acid yielded 32 g. of hydrazones. According to Anchel and Schoenheimer[4] the *p*-carboxyphenylhydrazones of α,β-unsaturated ketones are not split by formaldehyde, but are by pyruvic acid. Advantage was taken of this method to separate the ketones into a saturated and an unsaturated fraction.

Saturated Ketones.—On hydrolysis with formaldehyde[4] the total hydrazones yielded 12.7 g. of saturated ketones, which on fractional crystallization from ethanol and ether–methanol yielded a less soluble fraction which melted at 113–124°, and after adsorption on activated aluminum oxide and fractional elution with benzene was identified as cholestanone. A more soluble fraction had m. p. 78–79° unchanged on repeated recrystallization from ethanol, ether–methanol and acetone–ethanol. This

(1) This report is from a dissertation submitted by Herbert S. Anker in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

(2) Bloch and Rittenberg, *J. Biol. Chem.*, **149**, 505 (1943).

(3) Schoenheimer, Behring, Hummel and Schindel, *Z. physiol. Chem.*, **192**, 73 (1930).

(4) Anchel and Schoenheimer, *J. Biol. Chem.*, **114**, 539 (1936).

product [C, 83.6; H, 11.6; $[\alpha]_D$ +41.2° (1% in chloroform)] was believed to be a molecular compound of cholestanone and coprostanone into which it could be separated by the chromatographic method. The fraction was adsorbed from petroleum ether solution on a column of activated aluminum oxide. Fractional elution with petroleum ether–benzene 2:8 yielded coprostanone, which, after two recrystallizations from acetone–ethanol 1:3, melted at 60–61°. It gave no depression of m. p. when mixed with authentic coprostanone: $[\alpha]_D$ +36.2° (0.5% in chloroform).

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.9; H, 11.9. Found: C, 84.0; H, 11.6.

The fraction eluted with pure benzene was identified as cholestanone. After two recrystallizations from acetone–ethanol 1:1 it had m. p. 128.8–129.8°, unchanged on admixture of authentic cholestanone; $[\alpha]_D$ +42.7° (1% in chloroform).

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.9; H, 11.9. Found: C, 83.8; H, 11.6.

Unsaturated Ketones.—The hydrazones undecomposed by formaldehyde were treated with pyruvic acid[4] and yielded 2.4 g. of unsaturated ketones. These were dissolved in petroleum ether and adsorbed on activated aluminum oxide. Fractional elution with petroleum ether–acetone 19:1 gave 4,5-cholestenone, which, after two recrystallizations from acetone, melted at 79–80° and showed no depression of the m. p. with authentic 4,5-cholestenone; $[\alpha]_D$ +87.8° (1% in chloroform).

Anal. Calcd. for $C_{27}H_{44}O$: C, 84.4; H, 11.5. Found: C, 84.1; H, 11.2.

Alcohols.—From the non-ketonic residue (22.7 g.) the alcohols were separated as their hydrogen succinates by treatment with succinic anhydride in pyridine. After saponification 14.1 g. (37% of the total products) of alcohols was obtained, in a sample of which 84% was precipitable by digitonin. No crystalline alcohols could be isolated from the fraction not precipitable with digitonin. After two recrystallizations from acetone the material melted at 146–147°; $[\alpha]_D$ −36.1° (1.5% in chloroform).

Another sample of the alcohols was acetylated with acetic anhydride and the crude acetates adsorbed on activated aluminum oxide from petroleum ether solution and eluted with petroleum ether–benzene 9:1, after it had been established that under these conditions cholesterol and dihydrocholesterol acetates cannot be separated. 0.3584 g. = 0.8375 mM. of the eluted acetates was catalytically hydrogenated in glacial acetic acid solution. 20.9 ml. of hydrogen (760 mm.; 25.5°) was absorbed corresponding to 0.8266 mM., *i. e.*, 99% of the amount required for a sterol containing one double bond. Thus, there is no evidence that the alcoholic fraction contains any sterol other than cholesterol.

Hydrocarbons.—The 5.1 g. of material which remained after removal of the alcohols was passed through a column of activated aluminum oxide in petroleum ether solution. From the non-adsorbed material cholestane was obtained after repeated recrystallization from ethanol. It melted at 79.5–80.5° and did not depress the m. p. of authentic cholestane.

Anal. Calcd. for $C_{27}H_{48}$: C, 87.1; H, 12.9. Found: C, 86.8; H, 13.5.

The mother liquors remaining from the cholestane crystallizations resisted all attempts to obtain additional, well-characterized fractions. They were therefore combined and the solvent removed. The residue had a rotation of $[\alpha]_D$ +24.3° (1.5% in chloroform) and contained C, 87.5; H, 12.5. The probability that this residue is a mixture of cholestane and coprostane is supported by the rotation (cholestane, $[\alpha]_D$ +24.5°; coprostane, $[\alpha]_D$ +25.6°).

Action of Platinum and Dilute Acetic Acid on Cholesterol in the Presence of Deuterium

The fractions investigated had been obtained in an earlier experiment[2] in the following manner: 1.25 g. of platinum oxide in 40 ml. of acetic acid (60 atom % D) and 13 ml. of water (99 atom % D) were reduced by ordinary hydrogen and after addition of 12.5 g. of cholesterol shaken for three days at 127° in evacuated and sealed flasks. After saponification of the reaction mixture 5.5 g. of cholesterol was obtained by crystallization and precipitation with digitonin.

The combined mother liquors were separated by a method analogous to that described above.

Alcohols.—By treatment with succinic anhydride in pyridine an additional 1.1 g. of alcohols was obtained.

Ketones.—In the previous experiments with non-isotopic material it had been noted that the saturated and unsaturated ketones could be separated more conveniently by adsorption on activated aluminum oxide and fractional elution. In this experiment, therefore, the total *p*-carboxyphenylhydrazones were decomposed by pyruvic acid; 2.2 g. of ketones was obtained.

After adsorption on activated aluminum oxide fractional elution by petroleum ether–benzene 8:2 yielded 35 mg. of coprostanone (m. p. 58–60°), by benzene gave 60 mg. of cholestanone (m. p. 128–128.5°) and by petroleum ether–acetone 19:1, 30 mg. of 4,5-cholestenone (m. p. 77–78°) was obtained.

Hydrocarbons.—A solution of 0.8 g. of non-ketonic material in about 20 ml. of petroleum ether was passed through a column of activated aluminum oxide; 70 mg. of cholestane was obtained (m. p. 74–76°).

All mother liquors were combined. After evaporation of the solvent a semi-solid mixture of cholestane and coprostane remained.

The isotope analyses[5] of the various compounds are given in Table I.

Action of Platinum and Dilute Acetic Acid on Dihydrocholesterol

A preparation of dihydrocholesterol available in this Laboratory was used for this experiment. The recrystallized product melted at 140–142° and had a rotation of $[\alpha]_D$ +23.1° (1.5% in chloroform). The Liebermann–Burchard test was negative. On fractionation of the reaction products it became apparent that the "dihydrocholesterol"[6] employed was a mixture. A sample treated with digitonin yielded 73.3% of precipitable material. From the filtrates containing the non-precipitable material epicholestanol was obtained.

One gram of platinum oxide was reduced by hydrogen in 10 ml. of acetic acid; 9 g. of "dihydrocholesterol," 15 ml. of acetic acid and 25 ml. of water (6.4 atom % D) were added and the mixture shaken in an evacuated and sealed flask for one hundred hours at 127°.

The reaction products were separated in a manner analogous to that described before.

After saponification of the reaction mixture no acidic material could be obtained from the alkali layer.

Alcohols.—The alcoholic fraction separated by way of the hydrogen succinates weighed 5.8 g. (85% of the total products). A sample of the mixture of epimeric alcohols yielded 74.9% of digitonides. The ratio of cholestanol to epicholestanol was therefore identical with that of the starting material. Decomposition of the digitonides by pyridine and ether[7] yielded cholestanol (m. p. 140–141°, unchanged on admixture of an authentic sample). From the filtrate of the digitonides epicholestanol was obtained (m. p. 185–186°, unaltered by authentic epicholestanol).

Oxidation of Recovered "Dihydrocholesterol."—The mixture of the epimeric alcohols was oxidized with chromic acid according to the procedure of Windaus.[8] The ether soluble material was dissolved in 50 ml. of 2% ethanolic potassium hydroxide and refluxed for one hour in order

(5) Keston, Rittenberg and Schoenheimer, *J. Biol. Chem.*, **122**, 227 (1937).

(6) "Dihydrocholesterol" in the following refers to the mixture employed.

(7) Schoenheimer and Dam, *Z. physiol. Chem.*, **215**, 59 (1933).

(8) Windaus and Uibrig, *Ber.*, **47**, 2384 (1914).

to wash out any labile deuterium.[9,10] The cholestanone obtained was purified by adsorption on activated aluminum oxide and elution with benzene. The eluted material melted at 127–128°.

Ketones.—From the non-alcoholic material the ketones were separated as *p*-carboxyphenylhydrazones. These were decomposed by formaldehyde and yielded 0.45 g. of saturated ketones. No unsaturated ketone fraction was obtained. The ketonic material was adsorbed on activated aluminum oxide and fractionally eluted with petroleum ether–benzene 3:7. After recrystallization from acetone 220 mg. was obtained (m. p. 127–128°) which showed no depression of the m. p. with authentic cholestanone. There was no evidence of the presence of significant amounts of coprostanone.

Hydrocarbons.—The residue from which alcohols and ketones had been removed weighed 0.6 g. This fraction was passed through a column of activated aluminum oxide. The material washed out by petroleum ether was recrystallized from ethanol; 360 mg. of cholestane was obtained (m. p. 78–78.5°, not depressed by authentic cholestane). The absence of coprostane was indicated by the fact that pure cholestane was obtained after only one crystallization.

TABLE I

DEUTERIUM ANALYSES OF COMPOUNDS OBTAINED FROM THE CATALYTIC CHOLESTEROL EXCHANGE

Atoms D = (Atom % excess deuterium × number of hydrogen atoms in the compound)/100. Atoms D (calculated for 100% D) = (Atoms D × 100)/(Average ionizable D in medium), where average ionizable D in medium = (99 + 69.8)/2.

Compound	Excess deuterium, atom %	Deuterium, atoms	Deuterium (calculated for 100% D), atoms
Water used	99.0		
Acetic acid used	60.0		
Water recovered	69.8		
Acetic acid[a] recovered	54.0		
Cholesterol	4.16	1.91	2.3
4,5-Cholestenone	4.64	2.04	2.4
Cholestanone	7.47	3.44	4.1
Coprostanone	7.53	3.46	4.1
Cholestane	11.54	5.54	6.6
Cholestane + coprostane	10.67	5.12	6.0

[a] Analyzed as sodium acetate.

TABLE II

DEUTERIUM ANALYSES OF COMPOUNDS OBTAINED FROM THE CATALYTIC "DIHYDROCHOLESTEROL" EXCHANGE

Atoms D = (Atom % excess deuterium × number of hydrogen atoms)/100. Atoms D (calculated for 100% D) = (Atoms D × 100)/(Average ionizable D in medium), where average ionizable D in medium = (6.4 + 4.92)/2.

Compound	Excess deuterium, atom %	Deuterium, atoms	Deuterium (calculated for 100% D), atoms
Water used	6.4		
Water recovered	4.92		
"Dihydrocholesterol"	0.21	0.10	1.8
Epicholestanol	.34	.16	2.8
Cholestanol	.13	.06	1.1
Cholestanone[a]	.01	0	0
Cholestanone	.01	0	0
Cholestane	.48	.23	4.1

[a] By oxidation of "dihydrocholesterol" by chromic acid.

(9) Schoenheimer, Rittenberg and Graff, *J. Biol. Chem.*, **111**, 183 (1936).

(10) Anchel and Schoenheimer, *ibid.*, **125**, 23 (1938).

The values found by isotope analysis[5] are given in Table II.

Discussion

The identified products of the action of active platinum on cholesterol in dilute acetic acid were 4,5-cholestenone, 3-cholestanone, 3-coprostanone and cholestane. The presence of coprostane appeared probable, though its actual isolation was not realized.[11] The remaining alcoholic fraction was found to consist of pure cholesterol. Small amounts of acidic products were not identified.

The compounds isolated from the catalytic exchange of dihydrocholesterol were 3-cholestanone and cholestane. The remaining alcoholic fraction showed no change of steric configuration at carbon atom 5.

The nature of the reaction products suggests that a series of dehydrogenations and hydrogenations involving changes on carbon atoms 3, 4, 5 and 6 of cholesterol and on carbon atom 3 of dihydrocholesterol had occurred. The dehydrogenation of alcohols is known to proceed readily in the presence of heavy metal catalysts.[12,13] After saturation of the catalyst with hydrogen, dehydrogenation cannot proceed in a closed system unless the hydrogen is removed by acceptors. These chemical changes are promoted by the presence of the double bond since under the same conditions the destruction of cholesterol proceeds to a much greater extent than that of dihydrocholesterol. No hydrogenation of the double bond of cholesterol appears to occur under the conditions employed.

For the introduction of stably bound deuterium into the ring system and the side chain of the cholesterol molecule the double bond is essential. With dihydrocholesterol the exchange reaction is limited to carbon atoms 2, 3 and 4, since the samples of cholestanone, respectively, isolated and obtained by chromic acid oxidation of deuterio dihydrocholesterol, contained only normal hydrogen.

From the data in the earlier publication,[2] it can be calculated that in the cholesterol isolated, the exchange reaction had occurred with 2.3 atoms per molecule. Under the same conditions the isolated ketones and hydrocarbons formed as by-products of the exchange reaction have a higher deuterium content. In the experiment with dihydrocholesterol no deuterium is introduced into cholestanone and the isotope in cholestane can be accounted for by deuterium taken up as the result of hydrogenation. As, under the conditions employed, exchange of carbon-bound hydrogen with deuterium appears to occur only with cholesterol, the increased isotope content of the ketones and hydrocarbons in the cholesterol experiment is ascribable to deuterium uptake

(11) A similar finding has been reported by Windaus, *Ann.*, **453**, 101 (1924), who treated cholesterol with nickel at high temperatures in the absence of a solvent and found cholestanone and coprostanone in the reaction mixture.

(12) Liebig, *Chem. Zentr.*, 639 (1835).

(13) Badin, THIS JOURNAL, **65**, 1809 (1943).

resulting from specific chemical reactions rather than to generalized catalytic exchange.[14]

The quantities of cholestanone and coprostanone isolated after treatment of cholesterol were roughly equal and their deuterium concentrations were identical. This finding can best be explained by the assumption of a common precursor, both steric isomers being formed by a step involving the saturation of a double bond located at position 4–5 or position 5–6. This is in harmony with the fact that only cholestanone was found in the exchange reaction with dihydrocholesterol. Of the two possible precursors of cholestanone and coprostanone, *i. e.*, 4,5- and 5,6-cholestenone, only the 4,5 isomer could be isolated. The deuterium content of 4,5-cholestenone was about 2 atoms less than that of the saturated ketones. This excludes 4,5-cholestenone as precursor for the saturated ketones, because the deuterium introduced by hydrogenation at carbon atom 4 should be lost through enolization[15] and the difference in isotope content between the isolated 4,5-cholestenone and the saturated ketones should then be only 1 atom deuterium. On the other hand, the assumption that 5,6-cholestenone was the precursor of the saturated ketones accounts for the observed deuterium uptake.

Presumably 5,6-cholestenone was the primary dehydrogenation product of cholesterol and the isolated 4,5-cholestenone was formed from it by rearrangement, a reaction which occurs readily.[16] From the yields of the various ketones it may be inferred that the reduction of 5,6-cholestenone is a more rapid reaction than its rearrangement. The observation that the 4,5-cholestenone had a slightly higher deuterium concentration than the cholesterol suggests that some hydrogen exchange had occurred during the rearrangement.

The formation of both cholestane and coprostane and their identical isotope concentrations in the catalytic cholesterol exchange requires a common intermediate containing a double bond at carbon atom 5. In the absence of a double bond, only cholestane is formed. The isotope content of the hydrocarbons exceeds that of the saturated ketones by about 2 atoms of deuterium. This uptake of deuterium could be the result of reduction of cholestanone and coprostanone to cholestane and coprostane, respectively.

By another mechanism which could account for the saturated hydrocarbons, $\Delta^{3,5}$-cholestadiene or $\Delta^{2,4}$-cholestadiene would be formed as intermediates by dehydration of cholesterol with rearrangement of the double bond to the conjugate position in the case of the $\Delta^{2,4}$ isomer. The reduction of either intermediate would give rise to both cholestane and coprostane and would result in the required uptake of 4 atoms of deuterium. However, no unsaturated hydrocarbons could be isolated from the reaction mixture.

From the data reported here it is not possible to decide between these two possible mechanisms. It may be pointed out, however, that the conditions employed are not favorable for the dehydration of cholesterol. Anhydrous conditions and high temperatures are required for this reaction.[17] On the other hand, the catalytic reduction of ketones normally leads only to alcohols although in a few instances the formation of hydrocarbons has been observed.[18]

The deuterium content of cholestane formed in the exchange of dihydrocholesterol differs by about 2 atoms from that of the isolated alcohols. The responsible mechanism may be either one of the two discussed for the formation of hydrocarbons in the exchange of cholesterol.

The authors wish to express their gratitude to Dr. Hans T. Clarke for many valuable suggestions.

Summary

1. The products formed on treatment of cholesterol and of dihydrocholesterol with dilute acetic acid and active platinum at elevated temperatures were investigated.

2. Among the reaction products of cholesterol, 4,5-cholestenone, cholestanone, coprostanone, cholestane and coprostane were identified, whereas only cholestanone and cholestane were formed from dihydrocholesterol.

3. The experiments were repeated with a reaction mixture containing deuterium and the isolated products analyzed for isotope content. In contrast to the cholesterol exchange which results in a uniform distribution of deuterium over the whole molecule the treatment of dihydrocholesterol leads to introduction of deuterium only at positions adjacent to the hydroxyl group.

4. It is suggested that the primary dehydrogenation product of cholesterol, probably 5,6-cholestenone, is partly rearranged to 4,5-cholestenone and partly reduced to the isomeric saturated ketones. Two possible mechanisms accounting for the formation of the saturated hydrocarbons are discussed.

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(14) In order to calculate the number of deuterium atoms introduced into a compound, it is necessary to know the value of the equilibrium constant for the exchange reaction: $RH_n + D_2O \rightleftarrows RH_{n-1}D + HDO$, for in the catalyzed transfer of hydrogen from one compound to another the hydrogen will be equilibrated with the ionizable deuterium of the medium. The platinum catalyst employed in our experiments is extremely active in the water exchange reaction (private communication from Dr. D. Rittenberg). For random distribution the equilibrium constant for the reaction is 1, and actual measurements [Ingold and Wilson, *Z. Elektrochem.*, **44**, 62 (1938)] when RH_n is an organic compound, have in all cases given values close to 1. In our experiments we assume a random distribution of hydrogen and deuterium atoms and the calculations of the number of deuterium atoms introduced in the chemical reactions are based on this assumption. The numerical values are given in the last columns of Tables I and II.

(15) During the isolation procedure all fractions were subjected to repeated treatments with alkali. The saturated ketones exchange the hydrogen on carbon atoms 2 and 4 and 4,5-cholestenone the hydrogen on carbon atom 2 through enolization in alkaline medium.[9,10]

(16) Butenandt and Schmidt-Thomé, *Ber.*, **69**, 882 (1936).

(17) Mauthner and Suida, *Monatsh.*, **17**, 29 (1896).

(18) Hartung and Crossley, THIS JOURNAL, **56**, 158 (1934).