Molecular Rearrangements in the Sterols V. The Mechanism of Formation of *i*-Cholesterol

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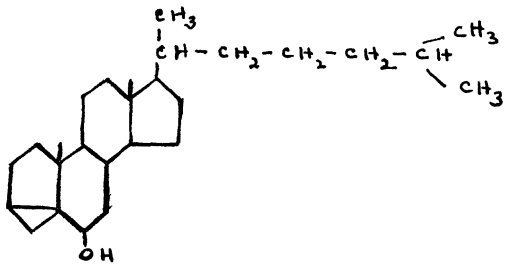
The present accepted structure of *i*-cholesterol (I) was first proposed by Wallis, Fernholz, and Gephart (*9*).



(I)

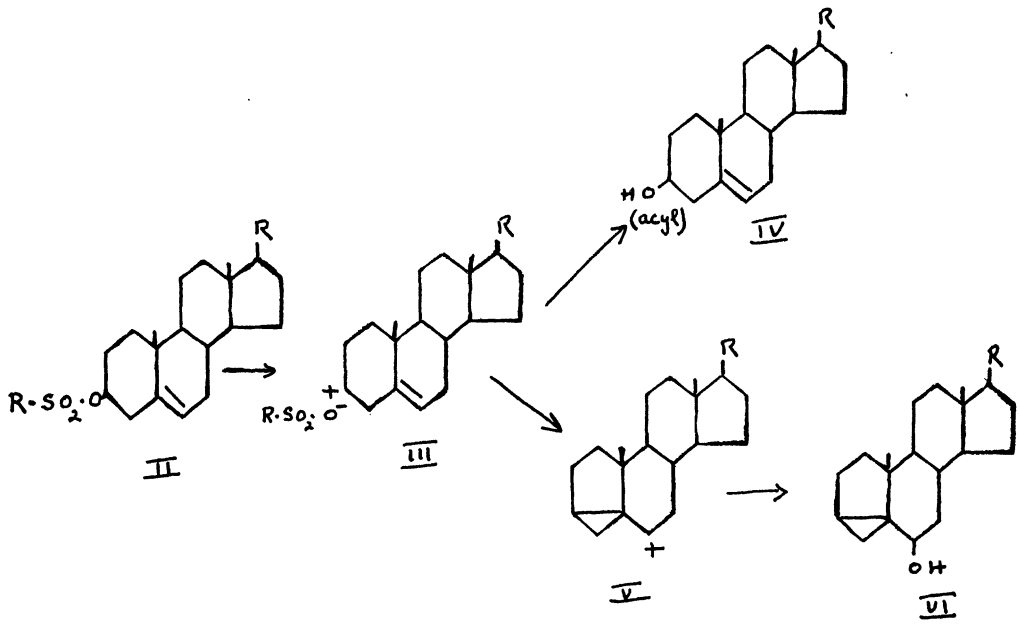
Proof of this formulation has been submitted by Ford, Chakravorty, and Wallis (*4*) and by Ladenberg, Chakravorty, and Wallis (*6*). These investigators (*4, 6, 9*) prepared mixed acetates of cholesterol and *i*-cholesterol by heating cholesteryl *p*-toluenesulfonate with acetic anhydride in the presence of potassium acetate. A mixture of the two isomers was also prepared by Benyon, Heilbron, and Spring (*1*) by hydrolysis of the same ester in aqueous acetone containing potassium acetate. In recent years it has been shown that the formation of *i*-steroids is a general property of all steroid compounds having a hydroxyl group in the β configuration at C_3 and a double bond at C_5—C_6 (*5*).

In this paper we wish to describe the results of certain studies we have made on the mechanism of this isomerization. For this purpose we have carried out the hydrolysis of a series of four sulfonic acid esters of cholesterol with different electron-donating powers in the acid residue. In order further to elucidate the mechanism we have varied the conditions of hydrolysis in the case of one of these esters—cholesteryl *p*-toluenesulfonate. The results of these studies are described in this paper.

From these results it is concluded that rearrangement of II is preceded by ionization—a conclusion in agreement with a mechanism recently proposed by Weinstein and Adams (*10*). The cholesteryl ion III is then either attacked by a negative ion and converted into the normal structure IV or it undergoes a rearrangement to the *i*-cholesteryl ion V, which is similarly converted to the isomeric sterol VI.

The first step of this process is slow. It is followed by a rapid conversion or rearrangement to the corresponding alcohols. On this concept the mechanism is, therefore, of the unimolecular type.

The breaking of the C—O linkage with ionization instead of the S—O linkage agrees with the work of Ferns and Lapworth (*3*) on the hydrolysis of sulfonic acid esters, and with the work of Phillips (*7*) on the Walden inversion of the *p*-toluenesulfonate of benzyl methyl carbinol. In the present instance we believe that our results show that the cholesteryl ion rearranges to the *i*-cholesteryl ion while still a free ion. This rearrangement may be looked upon as being the result of an electron shift at the double bond C_5—C_6 towards C_5, thereby binding the cationic center at C_3 and creating another cationic center at C_6. The negative hydroxyl ion or acetate ion, as the case may be, then attacks this new



center, forming *i*-cholesterol or its acetate. If such an attack occurs before the rearrangement of the cholesteryl ion then the normal product is produced.

It should be pointed out that this polarizability of C_5—C_6 double bond is in agreement with the interpretation of Shoppee (*8*) in explaining certain replacement reactions at the C_3 position of cholesterol, which takes place with retention of configuration while the same reactions with dihydrocholesterol result in a Walden inversion.

TABLE 1

PARTIAL HYDROLYSIS, UNDER IDENTICAL CONDITIONS, OF FOUR CHOLESTEROL ESTERS*

Ester	Wt in g	Wt of digitonide	% hydrolysis	K_1/sec
p-Methoxybenzene sulfonate	1.3920	1.2800	68.5	3.2×10^{-4}
p-Toluenesulfonate	1.3520	0.4992	87.1	5.7×10^{-4}
Benzenesulfonate .	1.3170	0.3968	90.1	6.7×10^{-4}
p-Nitrobenzene-sulfonate	1.4296	0.2976	92.6	7.2×10^{-4}

* One four-hundredth mole of the ester and 8/400ths mole of KOAc was refluxed for 1 hr at constant temperature with 50 ml of 90% aqueous acetone. The unhydrolyzed material was completely hydrolyzed with aqueous acetone and a very small amount of KOH. Cholesterol was precipitated as digitonide.

This unimolecular mechanism is also in accordance with the observation (*1, 4, 6*) that no trace of epicholesterol is detected, indicating that the replacing ion does not attach itself to the ester directly, but after ionization. Similarly, the very presence of rearrangement points to a unimolecular mechanism according to the views of Dostrosky and Hughes (*2*).

In Table 1 we have recorded results obtained in partial hydrolysis, under identical conditions, of four cholesteryl esters. The rates of hydrolysis show that the strength

TABLE 2

HYDROLYSIS OF THE FOUR ESTERS IN TABLE 1, CARRIED TO COMPLETION*

Ester	Wt of digitonide in g	% Conversion to *i*-cholesterol
p-Methoxybenzenesulfonate	0.4764	88.1
p-Toluenesulfonate	0.4840	87.9
Benzenesulfonate	0.4800	88.0
p-Nitrobenzenesulfonate	0.4832	87.9

* One four-hundredth mole of the ester and 8/400ths mole of KOAc were boiled for 3 hr with 50 ml of 70% aqueous acetone and then hydrolyzed with alcoholic KOH.

of the C—O bond is dependent, as expected, on the electron-donating power of the acid residue. The elimination of the sulfonate ion is facilitated by the weakened C—O bond, and is inversely proportional to the electron-donating power of the acid residue. Although these results can be reconciled with either a unimolecular or a bimolecular mechanism of hydrolysis, the fact that no traces of epicholesterol are formed strongly indicates that the replacing ion does not attack the ester directly and the reaction is consequently unimolecular in nature.

This point of view is definitely shown when the hydrolysis of the same four esters is carried to completion. In this case we find that the acid residue has no influence on the proportion of rearrangement, as is shown in Table 2. The rearrangement takes place in the cholesteryl ion after loss of the acid residue, which then has no effect on isomerization.

It is also of interest to observe the effect on isomerization of varying the water content of the hydrolyzing medium. This effect is shown in Table 3. Similarly, the influence of the potassium acetate concentration is shown in Table 4. Our results show that the lower the concentration of the active reagent, i.e., water and potassium acetate, the higher is the yield of *i*-cholesterol. Again this is understandable in the mechanism described above. The cholesteryl ion has a better chance to rearrange before the attack of the reagent.

TABLE 3

EFFECT ON ISOMERIZATION OF VARYING THE WATER CONTENT OF THE HYDROLYZING MEDIUM*

Concentration of water	Concentration of acetone	% *i*-Cholesterol
5%	95%	89.4
35%	65%	88.3
50%	50%	86.5

* One four-hundredth mole of cholesteryl *p*-toluenesulfonate was completely hydrolyzed in acetone solution in presence of 8/400ths mole of KOAc.

TABLE 4

EFFECT ON ISOMERIZATION OF VARYING THE POTASSIUM ACETATE CONCENTRATION*

Moles KOAc	% Conversion
2	89.5
8	87.9
32	87.0

* *p*-Toluenesulfonate was completely hydrolyzed in 80% aqueous acetone in presence of varying concentrations of KOAc.

There are, however, other alternatives for the rearrangement:

1. The rearrangement occurs concurrently with ionization. This case is very unlikely because the electron-donating power of the acid residue, which governs the ionization, does not affect the proportion of isomerization (Table 2).

2. The rearrangement takes place concurrently with addition of the negative ion at the moment of its attack. This case is unlikely because the proportion of isomerization is changed by changing the water or acetate concentration and it is not apparent how this change would affect isomerization taking place at the moment of attack by a single ion.

3. The cholesteryl ion, first formed, rearranges to form the *i*-cholesteryl ion, but (a) the rearrangement does not reach equilibrium, or (b) the rearrangement does effectively reach equilibrium but the proportion of the two ions is changed by a change in the medium.

It is not possible to eliminate possibilities (a) or (b) on the basis of the data presented here. However, if rearrangement of the cholesteryl ion can be thought of as an intramolecular reaction between an ion (the carbonium ion at C_3) and a neutral molecule (the double bond at C_5—C_6), alternative (b) can be eliminated, because there is no primary salt effect in such a reaction.

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Degradation of Glucose-1-C^{14} and a Possible New Step in the Mechanism of Fermentation[1]

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The availability of glucose-1-C^{14} [3] has permitted the verification of a scheme of glucose degradation applied to sugars formed in photosynthesis to determine the distribution of isotopic carbon within the sugar (*1*). As a result of the present investigation, there appears to be a second, though minor, pathway of fermentation by the test organism, *Lactobacillus casei* ε.

The degradation procedure is depicted in Fig. 1 and involves the following sequence of reactions: the fermentation of glucose to lactic acid; the oxidation of lactic acid to carbon dioxide (carbons three and four of the original glucose) and acetic acid; the pyrolysis of barium acetate to acetone and barium carbonate (carbons two and five); and the formation of iodoform (carbons one and six) from the acetone. The various steps in the process have been tested with (a) methyl- and carboxyl-labeled acetate, (b) α-labeled lactate,[4] and (c) glucose-1-C^{14}.

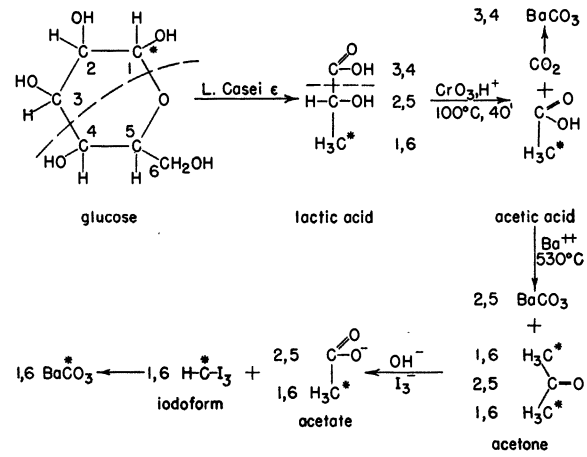

FIG. 1.

Both methyl- and carboxyl-labeled acetates have been pyrolyzed under a variety of conditions, a number of which can be so unfavorable (e.g., that at 450° C with flowing argon), as to result in the appearance of more than 8% of the activity of methyl-labeled acetate in the purified residual barium carbonate. Under similar conditions, with carboxyl-labeled acetate, less than ½ of 1% of the activity is in the iodoform. Using flowing argon, at a temperature of 530° C for 10 min, and liberating the carbon dioxide from the residual carbonate with lactic acid, only 0.86% of the activity of the methyl-labeled acetate is found in the carbonate.

Electrolysis of sodium acetate (*2*), the products of which are carbon dioxide and ethane, resulted in 1% of the radioactivity of the methyl group in the carbon dioxide from methyl-labeled acetate.

The chromium trioxide oxidation of α-labeled lactate (*7*) purified and recrystallized as the zinc salt, resulted in 4.3% of the activity in the carbon dioxide evolved. This activity arises primarily from oxidation of compounds other than the acetic acid itself, as less than 1% of the theoretical barium carbonate arises from acetic acid under identical conditions.

The degradation of lactate from the bacterial fermentation, similarly isolated and purified as the zinc salt, resulted in 9.3 ± 0.1% of the activity in the barium carbonate, rather than the empirical 4.3 ± 0.1. The difference of 5% must be ascribed to the activity of carbons three and four. In fermentation the carboxyl group of the lactic acid is presumed to arise solely from carbons three and four of glucose, whereas in ordinary chemical

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[3] The glucose used by the senior author was synthesized by John C. Sowden (*6*); that used in the California laboratory was synthesized by H. Mahler according to Sowden's general method (*4*).

[4] The acetates were synthesized by Dr. B. Tolbert, and the lactic acid by Dr. R. Lemmon (both at the University of California Radiation Laboratory).