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THE STEREOCHEMISTRY OF THE *i*-STEROIDS AND THEIR TRANSFORMATION PRODUCTS

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Although the gross structure of the steroid nucleus was elucidated in 1932 (1, 2), there has been a marked delay in the solution of many of the stereochemical aspects of the steroid structure. In particular, the stereochemistry of the replacement reactions at C-3 in steroids should be clarified. In this paper the configuration of the C-3 derivatives of cholesterol will be related to the configuration of the cholesterol by the use of stereospecific reactions, the formation and the rearrangement of the *i*-steroids. Then, after the determination of the configuration of groups at C-3 in steroids, a reasonable spacial configuration for the *i*-steroids will be postulated.

The replacement reaction at C-3 in steroids. The first extensive investigations of the replacement reaction in steroids were made by Marker (3) and by Ruzicka (4) while attempting to develop a feasible synthesis for androsterone. On the basis of a comparison of the melting points of the epimeric cholestyl chlorides with those of the epimeric cholestanols, Ruzicka suggested that a Walden inversion takes place in the formation of cholesteryl chloride from cholesterol. Bergmann (5), from a study of the replacement reaction on optically active halides with the acetate ion, took exception to this conclusion of Ruzicka. Bergmann contended that inversions take place on the treatment of the cholestyl chlorides with the acetate ion, and showed from this that the formation of cholesteryl chloride from cholesterol proceeds without inversion. Very recently Shoppee (6) has summarized the evidence on the replacement reaction in steroids and has reached the same conclusions as Bergmann on the basis of very similar evidence.

Decisive evidence that the conclusions reached originally by Bergmann are correct can be obtained from a study of the formation and rearrangement of the *i*-steroids. Some indication that the formation of *i*-cholesten-6-one (V) is stereospecific is apparent from the work of Windaus (7, 8). He found that "α"-chlorocholestan-6-one (IV) when warmed with alcoholic potassium hydroxide formed "heterocholestenone" (V), while "β"-chlorocholestan-6-one (IX) was recovered unchanged when treated under the same conditions. Since alkyl chlorides and alkyl *p*-toluenesulfonates show similar properties in replacement reactions, it was decided to study the reactions of "α" and "β"-chlorocholestan-6-one and to compare them with the reactions of the epimeric 3-*p*-toluenesulfonoxycholestan-6-ones. In this way a correlation between the configuration of the groups at C-3 of the various 6-ketocholestanes could be obtained, and from the methods of formation of these derivatives, a correlation

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between the configuration of cholesterol and cholesteryl chloride would also be established.

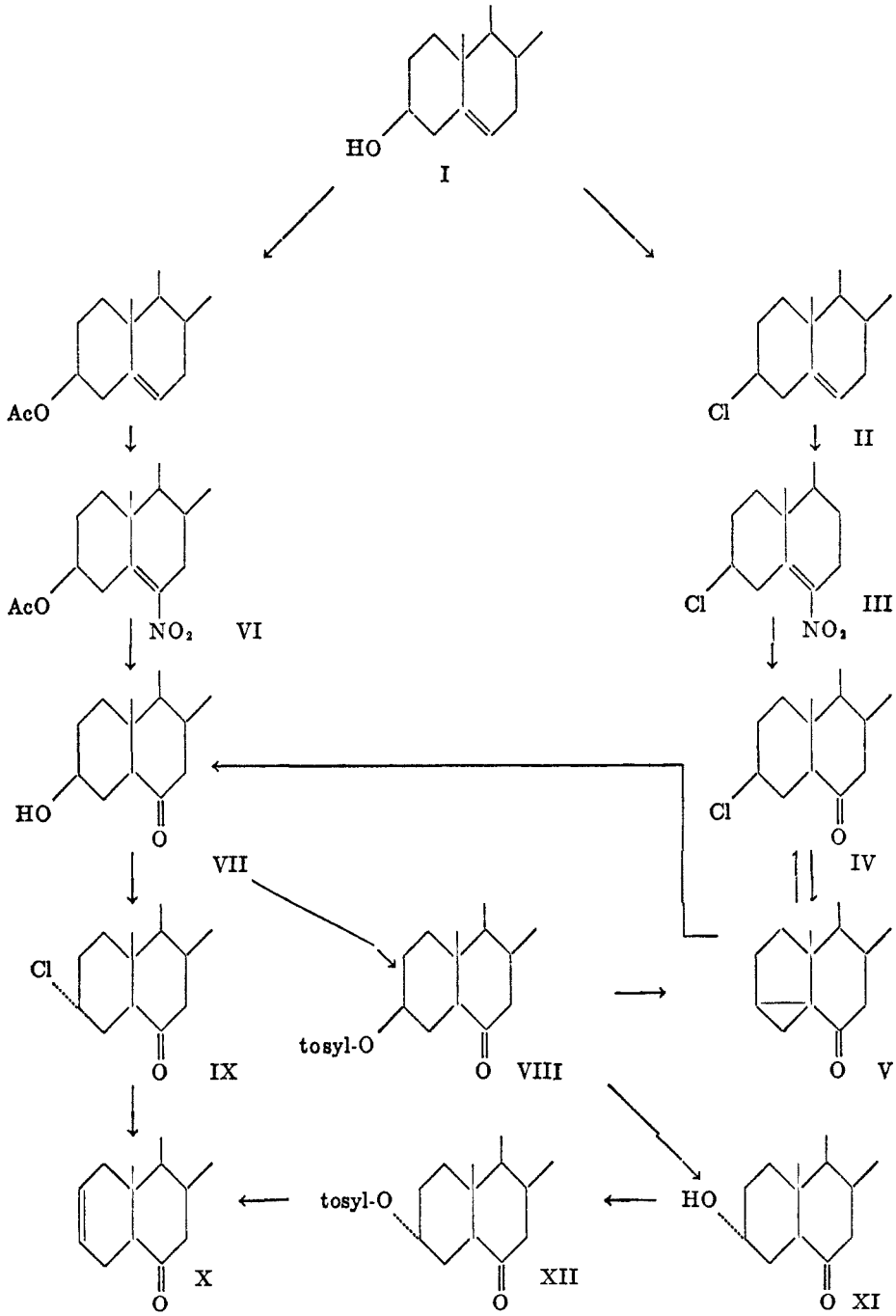
The stereochemical relationships confirmed by this work can be readily followed by reference to the chart of formulas. "α"-Chlorocholestan-6-one (IV) was easily obtained in good yield by the reduction of 6-nitrocholesteryl chloride (III) with zinc dust and acetic acid. 6-Nitrocholesteryl chloride (III), in turn, was prepared by the nitration of cholesteryl chloride (II) according to the method of Windaus and Dalmer (7). Thus in the preparation of "α"-chlocholestan-6-one from cholesteryl chloride, there is no possibility for inversion of the chloro group at C-3; that is, "α"-chlorocholestan-6-one has the same configuration at C-3 as cholesteryl chloride. On being heated for one hour in a 5% alcoholic potassium hydroxide solution, "α"-chlorocholestan-6-one (IV) was converted in 80–93% yield to *i*-cholesten-6-one (V) (7).

In order to prepare 3(β)-*p*-toluenesulfonoxycholestan-6-one (VIII), cholesteryl acetate was first nitrated to 6-nitrocholesteryl acetate (VI) in 69–78% yields according to the method of Mauthner and Suida (9). The nitro compound was then reduced with zinc dust and acetic acid to 3-acetoxycholestan-6-one and the acetate hydrolyzed to 3-hydroxycholestan-6-one (VII) with an alcoholic solution of hydrochloric acid. The over-all yield of 3-hydroxycholestan-6-one from 6-nitrocholesteryl acetate was 91%. This preparation of 3-hydroxycholestan-6-one differs from that developed by Heilbron (10), who experienced some difficulty in preparing 3-acetoxycholestan-6-one by the directions available in the literature.

In the preparation of 3-hydroxycholestan-6-one (VII) from cholesterol (I), it is necessary to first acetylate the C-3 hydroxyl group and then to hydrolyze the acetate. However, it is well established that acid catalyzed esterification of hydroxyl groups similar to this, as well as the hydrolysis of the resulting esters, does not cleave the alkyl-oxygen bond; only the acyl-oxygen bond is broken (11). Therefore, the configuration of the group at C-3 is left unchanged by these reactions, and compound VII is correctly formulated as 3(β)-hydroxycholestan-6-one.

This compound (VII) was easily converted to its *p*-toluenesulfonate by means of *p*-toluenesulfonyl chloride in anhydrous pyridine. Since the oxygen atom attached to C-3 must be that from the alcohol, it follows that the configuration of the tosylate at C-3 must also be the same as cholesterol. When heated in a 5% alcoholic potassium hydroxide solution, 3(β)-*p*-toluenesulfonoxycholestan-6-one (VIII) was converted in 85–92% yield to *i*-cholesten-6-one (V), identical with that obtained from "α"-chlorocholestan-6-one. From this, one can conclude that, if the formation of *i*-cholesten-6-one (V) is a stereospecific reaction, "α"-chlorocholestan-6-one (IV) has the same configuration as 3(β)-*p*-toluenesulfonoxycholestan-6-one. It would necessarily follow from this that cholesteryl chloride has the same configuration at C-3 as cholesterol. However, the assumption that the formation of *i*-cholesten-6-one is stereospecific must be proved.

In order to establish this, 3(β)-hydroxycholestan-6-one (VII) was converted to "β"-chlorocholestan-6-one (IX) according to the directions of Windaus and



Stein (12). It had previously been reported that this compound was recovered unchanged when heated with alcoholic potassium hydroxide (8). On repetition

of this experiment, it was found that 74% of the material was recovered unchanged after heating under reflux for one hour; the remaining, more soluble material, however, had a very low melting point, 75–85°. The "β"-chlorocholestan-6-one (IX) was, therefore, heated under reflux for nineteen hours with a 5% alcoholic solution of potassium hydroxide. Chromatography on alumina of the resulting mixture gave a 40–49% yield of 2-cholesten-6-one (X) and no other pure compound was isolated. Ladenburg, Chakravorty, and Wallis (13) were the first to describe the preparation of the unsaturated ketone (X). However, they assigned the ethylenic bond[2] to the 4,5-position in conjugation with the carbonyl group.

3(α)-Hydroxycholestan-6-one (XI) was prepared by the replacement with inversion of the tosylate group in 3(β)-*p*-toluenesulfonoxycholestan-6-one (VIII) by means of the acetate ion. The crude esters formed from this reaction were saponified, and the 2-cholesten-6-one, also formed in the reaction, was separated by crystallization from alcohol. The 3(α)-hydroxycholestan-6-one (XI) was then separated from the (β) isomer, which was also present, by chromatography on alumina. The structure of 3(α)-hydroxycholestan-6-one was proved by oxidation to cholestane-3,6-dione, identical with the compound obtained by the oxidation of the (β) isomer.

3(α)-*p*-Toluenesulfonoxycholestan-6-one (XII), prepared from the alcohol with *p*-toluenesulfonyl chloride in pyridine, was heated under reflux with a 5% alcoholic solution of potassium hydroxide in exactly the same way as had previously been done with the (β) tosylate. Since no pure product could be isolated by repeated crystallizations, the crude material was chromatographed twice on alumina. From the 1:1 benzene-petroleum ether eluate of the second chromatogram, a 23% yield of slightly impure 2-cholesten-6-one (X) was obtained.

From these elimination reactions, one can definitely conclude that the formation of *i*-cholesten-6-one is a stereospecific reaction. Both 3(β)-*p*-toluenesulfonoxycholestan-6-one (VIII) and "α"-chlorocholestan-6-one (IV) lose acid on treatment with alcoholic potassium hydroxide to form *i*-cholesten-6-one. The compounds epimeric with these at C-3 lost acid on similar treatment to form 2-cholesten-6-one. This naturally leads to the conclusion that 3(β)-*p*-toluenesulfonoxycholestan-6-one (VIII) and "α"-chlorocholestan-6-one (IV) possess the same configuration at C-3. Likewise, 3(α)-*p*-toluenesulfonoxycholestan-6-one (XII) and "β"-chlorocholestan-6-one (IX) must have the same configuration at C-3. It follows from this that no inversion has taken place in the formation of cholesteryl chloride from cholesterol. On the other hand, the action of phosphorus pentachloride on 3(β)-hydroxycholestan-6-one produces 3(α)-chlorocholestan-6-one with inversion. The trivial indices originally assigned to these chloro compounds must, therefore, be reversed. Cholesteryl chloride is actually

[2] The position of the carbon-carbon double bond in compound (X) was definitely established by Blunschy, Hardegger and Simon, *Helv. Chim. Acta*, **29**, 199 (1946). Although identical in all other respects, their compound did not display the ultraviolet absorption maximum at 2450 Å reported by the Princeton workers.

3(β)-cholesteryl chloride (II); "α"-chlorocholestan-6-one is 3(β)-chlorocholestan-6-one (IV); and "β"-chlorocholestan-6-one is actually 3(α)-chlorocholestan-6-one (IX).

The correlation of the optical rotatory powers of these compounds also indicates that no inversion takes place in the formation of cholesteryl chloride from cholesterol. Bernstein and co-workers (14) in a summary of their conclusions from an extensive study of the optical rotatory powers of steroids, state, "The C_3 (α)-form of any steroid will have a higher positive rotatory power (sodium light) than the corresponding (β)-form regardless of the solvent used." They indicate that the only exceptions to this rule may be in its application to $\Delta^{5,6}$-stenols in solvents other than chloroform. Our data on the optical rotatory powers of C-3 substituted derivatives of cholestan-6-one are summarized in Table I. Data on the epimeric chlorocholestanes and chloroandrostanes are included for comparison. It is seen that in all of the pairs of compounds with the exception of the epimeric dinitrobenzoates, the (α)-epimer has a higher positive rotation that the (β)-epimer. It appears, however, that the epimeric 3-(3,5-dinitrobenzoxy)cholestan-6-ones are an exception to this rule postulated by Bernstein.

TABLE I

SPECIFIC ROTATIONS

COMPOUND	SPECIFIC ROTATION, $CHCl_3$	
	(α)	(β)
3-Hydroxycholestan-6-one	+2.6	−5.1
3-Acetoxycholestan-6-one	−3.7	−15.5[a]
3-*p*-Toluenesulfonoxycholestan-6-one	+1.1	−5.5
3-(3,5-Dinitrobenzoxy)cholestan-6-one	−11.8	+1.7
3-Chlorocholestan-6-one	+7.7	−0.6
3-Chlorocholestane (6)	+30.5	+27
3-Chloroandrostan-17-one (6)	+94	+92

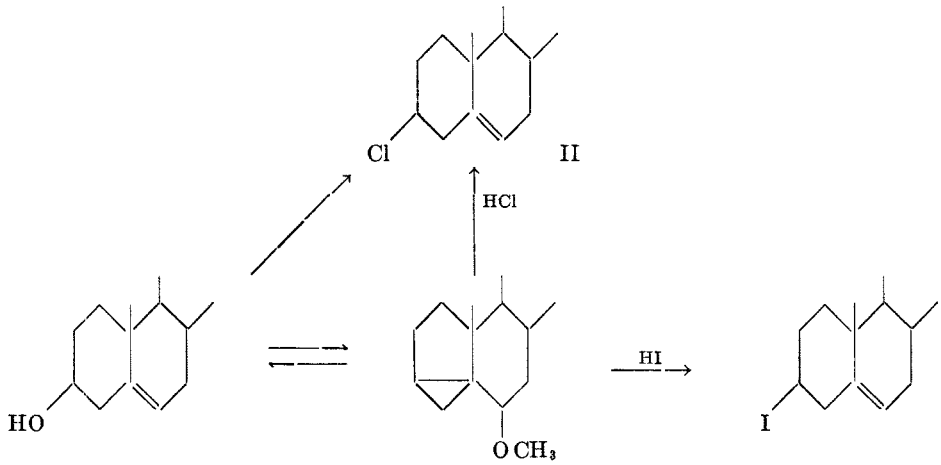
[a] Plattner and Lang, *Helv. Chim. Acta*, **27**, 1872 (1944), report the specific rotation of this compound in chloroform to be −15.2°; −13.8°.

The opening of the cyclopropane ring. The opening of the cyclopropane ring in *i*-cholesten-6-one also leads one to the above conclusions. Wallis and co-workers (13, 15) have shown that *i*-cholesten-6-one (V) is converted to "α"-chlorocholestan-6-one (IV) with hydrogen chloride in acetic acid and to 3(β)-hydroxycholestan-6-one (VII) with sulfuric acid in acetic acid, both in very good yields. Here the same stereospecific, acid-catalyzed reaction has taken place in both cases, and one can only conclude that the configurations of the compounds formed are identical.

The rearrangement of *i*-cholesteryl methyl ether (XIII) provides further evidence that the configurations of the hydroxyl group in cholesterol (I) and of the chloro group in cholesteryl chloride (II) are the same.[3] *i*-Cholesteryl methyl

[3] The correlation was independently recognized by Winstein and Adams, *J. Am. Chem. Soc.*, **70**, 838 (1948).

ether (XIII), formed from cholesteryl *p*-toluenesulfonate (16), is converted to the corresponding cholesteryl halides when treated with halogen acids in acetic acid (17). The yields are excellent, showing that the reaction is stereospecific; only one isomer is obtained. The cholesteryl chloride obtained from the rearrangement with replacement is identical with that prepared from cholesterol with phosphorus pentachloride. Similarly, the cholesteryl bromide obtained



is the same as that prepared with phosphorus tribromide. The *i*-cholesteryl methyl ether (XIII) is converted back to cholesteryl acetate[4] in good yield by refluxing it in acetic acid to which has been added a few drops of sulfuric acid. The yield for this conversion was about 80%. Since all of these reactions involve the same rearrangement with replacement, and since all of the compounds obtained are stereochemically pure, one can conclude that the configurations of all of the rearrangement products are identical. Thus the rearrangements of the *i*-steroids can be used to establish the configuration of C-3 of other sterol derivatives.

Recently, an independent determination of the configuration of the C-3 iodine in cholesteryl iodide (XIV), formed from *i*-cholesteryl ethyl ether, has been made by X-ray analysis (18). It was found that the carbon-iodine bond is *cis* to the methyl group at C-10. This, in conjunction with the preceding data, provides an independent proof of the (β) configuration of the hydroxyl group in cholesterol. This configuration was originally assigned by Ruzicka (19). It is also in agreement with Kendall's recent proof that the C-3 hydroxyl group in desoxycholic acid has an (α) configuration (20).

The spacial configuration of the i-steroids. The preceding information on the stereospecificity of *i*-steroid formation enables one to postulate a reasonable spacial configuration for *i*-cholesten-6-one. It has been shown by the Clemmensen reduction of cholestane-3,6-dione (21) to cholestane that the union of rings A and B in the 6-ketosteroids is *trans*; that is, the C-5 hydrogen in 3(β)-

[4] The authors are indebted to Dr. Yin-Lin Wang for this information.

chlorocholestan-6-one has an (α) configuration. It has been shown that *i*-cholesten-6-one is formed only from the (β)-chloride or tosylate. Thus in 3(β)-chlorocholestan-6-one, the hydrogen at C-5 and the chlorine at C-3 are *trans* to each other. Michael (22) has shown that it is easier to remove halogen acid from a *trans* olefinic derivative than from the *cis* isomer; hydrogen chloride is eliminated from chlorofumaric acid about forty-eight times as rapidly as from chloromaleic acid when they are treated under similar conditions with a base. It, therefore, appears that the hydrogen at C-5 and the chlorine at C-3 in 3(β)-chlorocholestan-6-one are ideally situated for elimination.

In an elimination reaction of this type, it is highly probable that the attack of the hydroxyl ion first results in the ionization of the C-5 hydrogen atom. The pair of electrons remaining at C-5 could then attack the back side of C-3, eliminating the chlorine atom with inversion at C-3. Models show that the compound so formed has the five-membered A ring and the six-membered B ring joined in a *cis* configuration. Hückel and co-workers (23) have shown that the *cis* configuration is the most stable configuration of the hexahydroindans. Carbon-4 in the cyclopropane ring is then *cis* to the methyl group at C-10. This model can be made without any undue strain except that which is normally associated with the cyclopropane ring.

On the other hand, if an attempt is made to eliminate hydrogen chloride from the 3,5-positions of 3(α)-chlorocholestan-6-one, either a *cis* elimination would be required or inversion of the carbanion must first take place at C-5 followed by a *trans* elimination. It has already been stated that *cis* elimination takes place with much greater difficulty than *trans* elimination. If inversion of the carbanion at C-5 takes place, followed by *trans* elimination, the five-membered A ring and the six-membered B ring would be joined in a *trans* configuration. The *trans* union in hexahydroindans results in a strained ring system in which the five-membered ring is no longer completely planar.[5] The cyclopropane ring, joined to a non-planar five-membered ring, would further increase the strain in this ring system. Considering these facts, one is not surprised that an isomeric *i*-cholesten-6-one has not been prepared by the 3,5-elimination of hydrogen chloride from 3(α)-chlorocholestan-6-one. Consideration of all of these facts also lends credence to the previous formulation of *i*-cholesten-6-one.

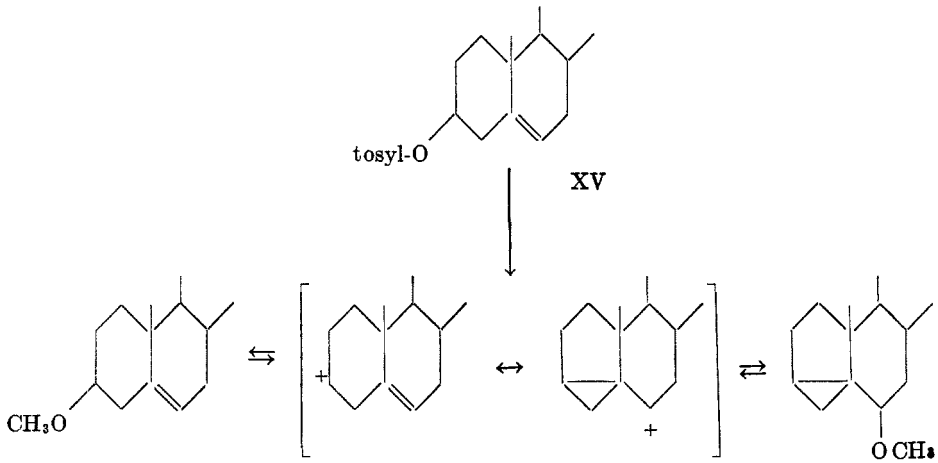
Wallis and co-workers (13, 15) and Heilbron and co-workers (24) have succeeded in converting *i*-cholesterol, formed by a rearrangement reaction, into *i*-cholesten-6-one, which is also formed by the 3,5-elimination reaction. Therefore, this same spacial configuration already assigned to *i*-cholesten-6-one should be assigned to all *i*-steroids irrespective of their method of preparation.

The mechanism of i-steroid formation and rearrangement. It should be emphasized that both the formation of *i*-cholesteryl methyl ether (XIII) from cholesteryl *p*-toluenesulfonate and the rearrangement of *i*-cholesteryl methyl ether to normal derivatives of cholesterol are stereospecific reactions. In all cases, only one of the two possible epimers has been isolated, and the yield of

[5] Consider, for example, the five-membered D ring in cholesteryl iodide, reference (18).

product is very high. Any mechanism for *i*-steroid formation and rearrangement must account for this stereospecificity of the reaction.

Stoll (16) first demonstrated that the reaction of cholesteryl *p*-toluenesulfonate (XV) with pure methanol leads to the formation of the normal methyl ether (XVII), while reaction in methanol containing sodium acetate or pyridine leads to the formation of the isomeric methyl ether, nor formulated, as XIII



XVII XVI XIII

above. Winstein[3] recently studied the kinetics of the reaction of cholesteryl *p*-toluenesulfonate (XV) with glacial acetic acid and found the reaction to be first order. Therefore, it is probable that in either case the first phase of the reaction of cholesteryl *p*-toluenesulfonate (XV) in methanol is the solvolysis of the tosylate to the hybrid cation (XVI), which can then react with methanol at either the C-3 or C-6 position to form the normal (XVII) or the isomeric (XIII) cholesteryl methyl ether respectively. Since in buffered solutions, where acid cleavage of either ether is eliminated, the product is primarily the *i*-methyl ether (XIII), the attack of methyl alcohol at C-6 of the hybrid cation must predominate over attack at C-3. In a buffered solution the reaction goes no further, and the predominant product is the *i*-steroid (XIII).

However, in acid solution a further reaction, the cleavage of the *i*-methyl ether, takes place. Since this ether is analogous to an allylic ether, cleavage by acids would be expected to proceed readily. Acid cleavage of the *i*-ether (XIII) would produce methanol and the hybrid cation (XVI), which would again be subject to attack by methanol at both C-3 and C-6. The normal methyl ether (XVII), on the other hand, is stable to dilute acids (17). Therefore, even though attack by methanol at C-6 predominates over attack at C-3, the normal methyl ether (XVII) would be the only product isolated from acid solution.

In order to explain the stereospecificity of these reactions, the structure of the resonance hybrid (XVI) must be examined. It is realized that the structures postulated for the resonance hybrid are not completely compatible with the

rules usually formulated for resonance hybrids (25). However, this hybrid (XVI) can be pictured as an ion with a partial but greatly distorted δ bond between C-3 and C-5 and a partial, distorted π bond between C-5 and C-6. In any case atomic orbitals from C-3 and C-6 are overlapping the orbital from C-5. If the configuration of the *i*-steroids is now considered, it follows that C-4 in the resonance hybrid (XVI) is already projecting up in a *cis* relationship to the C-10 methyl group and that the maximum electron density is on the (α) side of C-3. Attack by a nucleophilic reagent will of necessity take place at a position of minimum electron density; this must then be from the (β) side of C-3 and thus will account for the stereospecificity of *i*-steroid rearrangement.

Predication of the configuration of the methoxyl group at C-6 in *i*-cholesteryl methyl ether is difficult on the basis of this theory alone. However, from the structure of the resonance hybrid (XVI) it is clear that the π bond between C-5 and C-6 will be distorted and that this distortion will, most probably, lead to unequal electron densities on the (α) and (β) sides of C-6. The distortion of this π bond will, therefore, account for the stereospecificity of *i*-steroid formation. It is our opinion that the maximum electron density in the resonance hybrid is also on the (α) side of C-6, and that the methoxyl group at C-6 in *i*-cholesteryl methyl ether (XIII) has a (β) configuration.

This mechanism of *i*-steroid formation and rearrangement leads one to suspect that many replacement reactions at C-3 in $\Delta^{5,6}$ unsaturated steroids may go through an *i*-steroid intermediate. In any case, replacement reactions at C-3 cannot be discussed without considering the possibility of *i*-steroid formation and rearrangement.

Acknowledgment. The authors wish to express their appreciation to many former associates and friends that have contributed to this problem, especially Drs. Edwin W. Meyer, Samuel Siegel, and Saul Winstein.

EXPERIMENTAL[6]

6-Nitrocholesteryl acetate (VI). This compound was prepared in 70–78% yield by the nitration of cholesteryl acetate according to the directions given by Mauthner and Suida (9). Equal weights of cholesteryl acetate and sodium nitrite were always used.

3(β)-Hydroxycholestan-6-one (VII). 6-Nitrocholesteryl acetate (20 g.) was dissolved in 400 ml. of glacial acetic acid stirred with a Hershberg stirrer. This solution was diluted with 40 ml. of water. Then 40 g. of zinc dust was added to the solution in small portions over a period of four hours. After the initial exothermic reaction had subsided (one-half hour), the suspension was heated under reflux for the remaining reaction time (three and one-half hours). The solution was then filtered and the residue washed with two 25-ml. portions of acetic acid. The filtrate was diluted with 400 ml. of water, then cooled in an ice-bath. The precipitated 3(β)-acetoxycholestan-6-one was collected by filtration. To hydrolyze the acetate, the precipitate was dissolved in 200 ml. of ethanol, 60 ml. of concentrated hydrochloric acid was added, and the resulting suspension was heated under reflux for one hour. The suspension was then cooled to 5°, filtered, and the product washed with two 50-ml. portions of cold (5°) 70% ethanol. From this reduction 15.5 g. (91%) of

[6] We are indebted to Margaret Ledyard and Patricia Craig for the microanalyses reported in this paper. All melting points were taken on a Fisher-Johns melting point apparatus.

3(β)-hydroxycholestan-6-one, m.p. 140–141.5°, was obtained. Crystallized twice from methanol, the product melted at 142–143°, $[\alpha]_D^{24}$ −5.1±1° (93.3 mg. made up to 5 ml. with chloroform, α, −0.189°; *l*, 2 dm.). Mauthner (26) has reported the specific rotation −3.14° for this compound at 20° in ether.

3(β)-Acetoxycholestan-6-one. This acetate can be obtained directly by the crystallization from ethanol of the crude acetate isolated in the above reaction. It was also prepared by the acetylation of 3(β)-hydroxycholestan-6-one with acetic anhydride in pyridine. Crystallized twice from methanol, it melted at 127–128°, $[\alpha]_D^{24}$ −15.5±1° (82.0 mg. made up to 5 ml. with chloroform, α, −0.507°; *l*, 2 dm.).

3(β)-p-Toluenesulfonoxycholestan-6-one (VIII). 3(β)-Hydroxycholestan-6-one (5.0 g.) was dissolved in 11 ml. of anhydrous pyridine, and 5.0 g. of pure *p*-toluenesulfonyl chloride was added to the solution. The reaction mixture was warmed slightly on a steam-bath until homogeneous, then allowed to stand overnight at room temperature. The tosylate was isolated in the usual way. After crystallization from acetone, 6.2 g. (90%) of 3(β)-*p*-toluenesulfonoxycholestan-6-one was obtained. The melting point of this compound varies from 169° to 179° with the rate of heating of the melting point block; the melt becomes bright red very quickly. The pure compound, however, never melts over more than a 2° range, $[\alpha]_D^{21}$ −5.5±0.7° (75.6 mg. made up to 5 ml. with chloroform, α, −0.165°; *l*, 2 dm.).

Anal. Calc'd for $C_{34}H_{52}O_4S$: C, 73.34; H, 9.41.
Found: C, 73.55; H, 9.51.

i-Cholesten-6-one (V) from 3(β)-p-toluenesulfonoxycholestan-6-one (VIII). A solution of 2.00 g. of 3(β)-*p*-toluenesulfonoxycholestan-6-one in 100 ml. of 5% alcoholic potassium hydroxide was heated under reflux for one hour. The solution turned a light yellow. This solution was poured into 200 ml. of cold water, the resutling suspension stirred thoroughly, and the product separated by filtration. From the reaction 1.18 g. (85%) of *i*-cholesten-6-one, m.p. 96.5–97.5°, was obtained. Crystallization from alcohol raised the melting point to 97–98°, $[\alpha]_D^{22}$ 45.6±0.7°; 44.6±0.5° (54.2 mg. made up to 5 ml. with chloroform, α +0.989°; 122.5 mg. made up to 5 ml. with chloroform, α, +2.181°; *l*, 2 dm.). Heilbron (24) has reported the specific rotation 40.9° for *i*-cholesten-6-one at 18° in chloroform solution. A mixture of this compound with a sample of *i*-cholesten-6-one prepared from 3(β)-chlorocholestan-6-one showed no melting point depression. Yields as high as 92% of *i*-cholesten-6-one have been obtained from this *p*-toluenesulfonate.

3(β)-Chlorocholestan-6-one (IV) was prepared according to the method of Windaus and Dalmer (7). Recrystallized from ethanol, the compound melted at 129.5–130.5°, $[\alpha]_D^{24}$ −0.6±0.6° (85.8 mg. made up to 5 ml. with chloroform, α, −0.021°; *l*, 2 dm.). When heated in a 5% alcoholic solution under reflux for one hour, *i*-cholesten-6-one, m.p. 96–97°, was formed in yields ranging from 79–93%.

3(α)-Chlorocholestan-6-one (IX). 3(β)-Hydroxycholestan-6-one, when treated with phosphorus pentachloride according to the direction of Windaus and Stein (12), is converted to 3(α)-chlorocholestan-6-one, m.p. 181.5–182.5°, $[\alpha]_D^{24}$ 7.7±0.3° (98.3 mg. made up to 5 ml. with chloroform, α, +0.304°; *l*, 2 dm.).

Reaction of 3(α)-chlorocholestan-6-one (IX) with alcoholic potassium hydroxide. A solution of 0.50 g. of 3(α)-chlorocholestan-6-one in 75 ml. of 5% alcoholic potassium hydroxide was heated under reflux for one hour. The solution turned light yellow in color. The boiling solution was diluted with water until cloudy, cooled in ice, and the product separated from the solution by filtration. The product was washed thoroughly on the filter with cold methanol. From this reaction 0.37 g. (74%) of the starting material, m.p. 176.5–179.5°, was recovered. The mother liquors from the above crystallization were diluted with water, and the solid that precipitated was separated by filtration, m.p. 75–85°.

In a second experiment a solution of 0.50 g. of 3(α)-chlorocholestan-6-one in 75 ml. of 5% alcoholic potassium hydroxide was heated under reflux for nineteen hours. The resulting yellow solution was diluted with water and thoroughly extracted with ether. The ether solution was washed twice with water then evaporated to dryness. The residual yellow syrup was dried over phosphorus pentoxide under vacuum. It was then dissolved

in 30 ml. of a 1:1 mixture of benzene and petroleum ether (Skellysolve B, b.p. 60–70°) and chromatographed on a 2.5 x 12.5 cm. column of activated alumina (40 g.). The first fraction eluted with 50 ml. of a 1:1 benzene-Skellysolve B mixture contained 0.184 g. (40%) of 2-cholesten-6-one, m.p. 104.5–105.5°. No pure products were obtained from any of the other eluates. The 2-cholesten-6-one was converted to its oxime by heating with hydroxylamine hydrochloride and pyridine for one hour, m.p. 187.5–190° after crystallization from alcohol. This oxime when prepared from a sample of 2-cholesten-6-one, m.p. 103.5–104.5°, that was not chromatographically pure melted at 183.5–185.5°. Wallis (13), who first prepared 2-cholesten-6-one from 3(β)-bromocholestan-6-one, reports that the compound melts at 104–105° and its oxime melts at 184–185°. In other experiments yields of 2-cholesten-6-one as high as 49% have been obtained.

3(α)-Hydroxycholestan-6-one (XI). A solution of 2.00 g. of 3(β)-*p*-toluenesulfonoxycholestan-6-one (VIII) and 5.0 g. of anhydrous sodium acetate in 25 ml. of glacial acetic acid was heated under reflux for seventeen hours. The resulting solution was diluted with water and thoroughly extracted with ether. The ether solution was washed first with water, then with a dilute solution of sodium bicarbonate, and finally with water. The residue obtained on evaporation of the ether was saponified by heating it under reflux for one hour with 25 ml. of 10% alcoholic potassium hydroxide. The hot, alcoholic solution was then diluted with water until cloudy and cooled in ice. The precipitate that formed was separated by filtration and washed on the filter with 8 ml. of cold methanol. In this way 0.62 g. (45%) of crude 2-cholesten-6-one, m.p. 95–100°, was isolated. One crystallization of this product from ethanol gave relatively pure 2-cholesten-6-one, m.p. and mixed m.p. 102.5–104.5°.

The mother liquors from the isolation of 2-cholesten-6-one were heated to boiling and diluted with hot water until rather cloudy. They were then cooled to 0° and the precipitate that formed was separated by filtration. From a crystallization of this precipitate from dilute methanol, 0.39 g. of the crude alcohols, m.p. 110–123°, were isolated. This material was dried thoroughly over phosphorus pentoxide under vacuum. It was dissolved in 25 ml. of benzene and chromatographed on 40 g. of alumina in a column 2.5 x 12 cm. The column was first washed free of any unsaturated material with benzene, then developed with a mixture of Skellysolve B and acetone (3:1). The Skellysolve B-acetone eluates were evaporated; the fractions melting above 155° were combined and crystallized from dilute methanol. From this reaction 0.151 g. (10.4%) of 3(α)-hydroxycholestan-6-one, m.p. 159–160.5°, was obtained; $[\alpha]_D^{24}$ 2.6±0.9° (85.3 mg. made up to 5 ml. with chloroform, α, +0.090°; l, 2 dm.).

Anal. Calc'd for $C_{27}H_{46}O_2$: C, 80.55; H, 11.52.
Found: C, 80.26; H, 11.82.

In order to determine whether the 2-cholesten-6-one was formed during the replacement reaction or whether it was formed in the subsequent hydrolysis, the product from a second replacement reaction was chromatographed on alumina before saponification of the esters. In this way 0.55 g. (18%) of pure 2-cholesten-6-one, m.p. 103–104.5°, was obtained showing that the 2-cholesten-6-one was definitely formed during the replacement reaction. The crude alcohols were isolated from the remaining fractions after saponification, and chromatographed as before. The Skellysolve B-acetone (4:1) eluate yielded 0.433 g. (13.5%) of 3(α)-hydroxycholestan-6-one, m.p. 160–161° after crystallization from dilute methanol. The final fraction eluted from the column with acetone after two crystallizations from methanol gave slightly impure 3(β)-hydroxycholestan-6-one, m.p. 130–135°. This compound was converted in the usual way to 3(β)-*p*-toluenesulfonoxycholestane-6-one, m.p. and mixed m.p. 174.5–176°. It is interesting to note that the epimeric hydroxycholestanones can be separated relatively easily by chromatography on alumina; fractional crystallization of either the free alcohols or the *p*-toluenesulfonates was completely ineffective in achieving this separation.

Cholestane-3,6-dione from 3(α)-hydroxycholestan-6-one (XI). A chromic acid solution was made by the addition of 8 g. of concentrated sulfuric acid and 6 g. of crystalline sodium

dichromate to 27 g. of water (27). To a solution of 42.6 mg. of 3(α)-hydroxycholestan-6-one in 0.5 ml. of acetic acid, 0.085 ml. of the chromic acid solution was added. The resulting mixture was warmed in a water-bath at 70° for fifteen minutes. The suspension was then diluted with water, filtered, and the product washed thoroughly with water. The cholestane-3,6-dione so obtained was crystallized from dilute alcohol and washed on the filter with cold methanol. From this oxidation 32.8 mg. (77%) of cholestane-3,6-dione, m.p. 168.5–170°, was obtained. Crystallization from dilute acetic acid raised its melting point to 169.5–170.5°. A mixture with the cholestane-3,6-dione prepared from 3(β)-hydroxycholestan-6-one, according to the method of Windaus (28), showed no depression in melting point.

3(α)-p-Toluenesulfonoxycholestan-6-one (XII). To a solution of 200 mg. of 3(α)-hydroxycholestan-6-one in 1 ml. of anhydrous pyridine was added 200 mg. of pure *p*-toluenesulfonyl chloride. The mixture was warmed on the steam-bath until homogeneous, then allowed to stand overnight at room temperature. The product was isolated in the usual way, then crystallized from methanol to give 162 mg. (59%) of 3(α)-*p*-toluenesulfonoxycholestan-6-one, m.p. 145.5–147°. A second crystallization from methanol raised its melting point to 147–148°, $[\alpha]_D^{24.6}$ 1.1±0.5° (89.3 mg. made up to 5 ml. with chloroform, α, +0.038°; *l*, 2 dm.). In a second preparation of this tosylate a 68% yield was obtained.

Anal. Calc'd for $C_{34}H_{52}O_4S$: C, 73.34; H, 9.41.
Found: C, 73.47; H, 9.32.

Reaction of 3(α)-p-toluenesulfonoxycholestan-6-one (XII) with alcoholic potassium hydroxide. A solution of 50.0 mg. of 3(α)-*p*-toluenesulfonoxycholestan-6-one in 4 ml. of 5% alcoholic potassium hydroxide was heated under reflux for one hour. The clear solution immediately turned light yellow. After one hour, the solution was diluted with water and the product extracted with ether. The ether solution was washed thoroughly with water and evaporated to dryness. The residue was thoroughly dried under vacuum over phosphorus pentoxide. Since preliminary experiments had shown that no pure product could be obtained by crystallization of this residue, it was dissolved in 5 ml. of Skellysolve B and chromatographed on 10 g. of alumina in a column 1.2 x 12.5 cm. The fraction eluted with benzene weighed 16.9 mg. (49%) m.p. 83–90°. This fraction was chromatographed a second time on 5 g. of alumina. The fraction eluted with a mixture of Skellysolve B and benzene (1:1) consisted of 7.8 mg. (23%) of slightly impure 2-cholesten-6-one, m.p. 98–101°. A mixture with an authentic sample of 2-cholesten-6-one melted at 101–104°. A mixture with a portion of *i*-cholesten-6-one melted below 75°. Further eluates yielded only impure material.

3(α)-Acetoxycholestan-6-one was made by the acetylation of 3(α)-hydroxycholestan-6-one with acetic anhydride in anhydrous pyridine. After crystallization from dilute methanol, the product melted at 107–108°, $[\alpha]_D^{21}$ −3.7±1° (43.8 mg. made up to 5 ml. with chloroform, α, −0.064°; *l*, 2 dm.).

Anal. Calc'd for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88.
Found: C, 78.56; H, 10.95.

3(α)-(3,5-Dinitrobenzoxy)cholestan-6-one was made by treating a solution of 68.1 mg. of 3(α)-hydroxycholestan-6-one in 3 ml. of pyridine with 140 mg. of 3,5-dinitrobenzoyl chloride, m.p. 66–67°. From the reaction 94.5 mg. (94%) of product, m.p. 175.5–176.5°, was obtained. Crystallization from dilute acetone failed to raise the melting point, 175.5–176.5°, $[\alpha]_D^{24}$ −11.8±0.7° (76.7 mg. made up to 5 ml. with chloroform, α −0.362°; *l*, 2 dm.).

Anal. Calc'd for $C_{34}H_{48}N_2O_7$: C, 68.43; H, 8.11; N, 4.70.
Found: C, 68.25; H, 8.20; N, 4.58.

3(β)-(3,5-Dinitrobenzoxy)cholestan-6-one was prepared from 3(β)-hydroxycholestan-6-one according to the directions given by Shriner and Fuson (29) for the preparation of dinitrobenzoates. After crystallization from acetone, the compound melted at 234–235°, $[\alpha]_D^{24}$ +1.7±0.4° (72.9 mg. made up to 5 ml. with chloroform, α, +0.050°; *l*, 2 dm.).

Anal. Calc'd for $C_{34}H_{48}N_2O_7$: C, 68.43; H, 8.11; N, 4.70.
Found: C, 68.74; H, 8.30; N, 4.84.

SUMMARY

It has been shown that the formation of *i*-cholesten-6-one is a stereospecific reaction. Both 3(β)-*p*-toluenesulfonoxycholestan-6-one and 3(β)-chlorocholestan-6-one form *i*-cholesten-6-one on treatment with alcoholic potassium hydroxide. The (α)-isomers form 2-cholesten-6-one on similar treatment.

From the methods of preparation of 3(β)-*p*-toluenesulfonoxycholestan-6-one and 3(β)-chlorocholestan-6-one, and the stereospecificity of the formation of *i*-cholesten-6-one, it was concluded that no inversion of configuration is involved in the formation of cholesteryl chloride from cholesterol.

Further data justifying this assignment of configuration were obtained from the specific rotations of the compounds.

It was indicated that the transformation of either *i*-cholesteryl methyl ether or *i*-cholesten-6-one to normal steroids results in a (β) configuration of the groups at C-3.

The above conclusions in conjunction with the complete X-ray analysis of cholesteryl iodide provide independent proof that the C-3 hydroxyl group in cholesterol has a (β) configuration.

On the basis of the known configuration of 3(β)-chlorocholestan-6-one and 3(β)-*p*-toluenesulfonoxycholestan-6-one, a spacial configuration has been assigned to *i*-cholesten-6-one.

A mechanism has been postulated for the formation and rearrangement of the *i*-steroids that accounts for the stereospecificity of the reaction. It has been suggested that many of the replacement reactions at C-3 in cholesterol go through an *i*-steroid intermediate.

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