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ACID-CATALYSED DEHYDRATION OF 3-HYDROXYSTEROIDS—II

THE INFLUENCE OF SOLVENTS ON THE PRODUCTS OF THE REACTION BETWEEN HYDROGEN CHLORIDE AND 3β-HYDROXY-Δ⁵-STEROIDS

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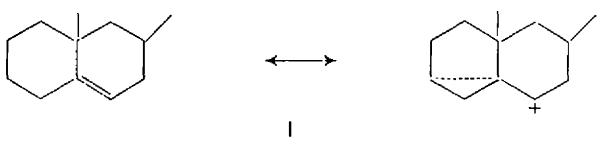
Abstract—Investigation of the products obtained from reaction of cholesterol with hydrogen chloride has shown that in some solvents (e.g. di-isopropyl ether) yields are low and substitution products are dominant whilst in others (e.g. ethanol) yields are higher and elimination products predominate. Hydrogen chloride in 2-chloroethanol is unexpectedly reactive with cholesterol. The formation of the elimination product, cholesta-3,5-diene (IV), by acid-catalysed dehydration of cholesterol has been shown to proceed by a route which does not involve the carbonium ion (I). The results are interpreted by postulating either a unimolecular or a bimolecular elimination in competition with unimolecular substitution. It is considered that the varying proportions in which the products of the competing reactions are formed depends primarily on the extent of the solvent stabilization of the protonated steroidal alcohol.

DURING a study[1] of the isolation of diosgenin and other steroid sapogenins from Uganda *Dioscorea sp.* it was shown that the acid-catalysed hydrolysis of the saponins was accompanied by dehydration of some of the sapogenin. Furthermore it was shown that 3β-hydroxy-Δ⁵-steroids in general undergo dehydration on treatment with ethanolic acid to form a steroidal 3,5-diene.

A unimolecular elimination process for such an acid-catalysed dehydration process would be expected to pass through the mesomeric carbonium ion intermediate (I).



I

The formation of this ion is well established in the solvolysis[2] of the toluene-*p*-sulphonates of 3β-hydroxy-Δ⁵-steroids and the acid-catalysed rearrangements of 3,5-cyclosteroids, e.g. the formation of 3β-ethoxycholest-5-ene (II, R = OC_2H_5) from 6β-methoxy-3α,5-cyclo-5α-cholestane (III) proceeds through this mesomeric carbonium ion[3] (I). When 6β-methoxy-3α,5-cyclo-5α-cholestane (III), in refluxing ethanol, was treated with ethanolic hydrogen chloride (overall acid concentration 2M) for 3 min, rearrangement was quantitative and rapid. 3β-Chlorocholest-5-ene

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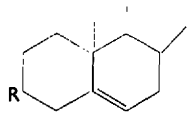
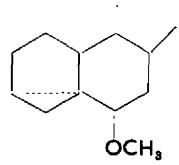
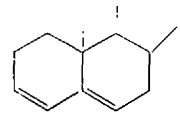
[1] W. J. Peal, *Chem. & Ind.* 1451 (1957). (Paper I in this series).
[2] L. F. Fieser and M. Fieser, *Steroids* p. 316. Reinhold, New York (1959).
[3] S. Winstein and A. H. Schlesinger, *J. Amer. Chem. Soc.* **70**, 3528 (1948).



II



III



IV

(II, R = Cl, 58·5%) and 3β-ethoxycholest-5-ene (II, R = OC_2H_5, 37·5%) were isolated. There was no detectable cholesta-3,5-diene (IV) or 3β-methoxycholest-5-ene (II, R = OCH_3). On the other hand, when cholesterol was treated for 6 hours under similar conditions, a mixture of cholesta-3,5-diene (IV, 29·7%), 3β-chlorocholest-5-ene (II, R = Cl, 7·4%) and 3β-ethoxycholest-5-ene (II, R = OC_2H_5, 2·5%) was obtained. It is possible that small amounts of other primary reaction products may also have been formed. The comparative slowness of this reaction and the dominant production of cholesta-3,5-diene (IV) indicates that elimination proceeds by a route which does not involve the carbonium ion I.

Attempts to determine the mechanism of the dehydration reaction by kinetic experiments were unsuccessful since, at the temperature required for relatively rapid reaction, the acid concentration decreases due to the formation of alkyl chlorides. Moreover cholesta-3,5-diene (IV) slowly reacts in the presence of hydrogen chloride. An alternative approach to the problem was therefore chosen, namely the investigation of the products of the reaction between cholesterol and hydrogen chloride in differing solvents under standard conditions. The present paper presents the results of this study together with some further experiments which enable possible mechanisms for the dominant elimination reaction to be discussed. The results of the reactions of 3α-methylcholesterol under similarly varied conditions are presented in the following paper.[4]

It was found that, for many solvents, a 2M concentration of hydrochloric acid reacting with cholesterol at 80° for 6 hours gave products in sufficient yields to be analysed by alumina chromatography. These were therefore adopted as standard conditions. It was not found possible to separate completely cholesta-3,5-diene (IV) from 3β-chlorocholest-5-ene (II, R = Cl) by column chromatography on alumina or other adsorbents. The fractions containing these two compounds were analysed for cholesta-3,5-diene (IV) by determination of the extinction coefficient at 236 mμ and for 3β-chlorocholest-5-ene (II, R = Cl) by chlorine analysis after detection of the presence of the chloride from an IR spectrum. The presence of 3β-chlorocholest-5-ene (II, R = Cl) was confirmed in a number of cases by solvolysis to cholesterol.[5] When it was found that the yields did not enable satisfactory comparison to be made under the standard reaction conditions the procedure was modified and the products of the reaction compared, wherever possible, with the products of the reaction of cholesterol and 2M ethanolic hydrogen chloride similarly modified. Results of these experiments are shown in Table 1.

It has also been found that when 3β-ethoxycholest-5-ene (II, R = OC_2H_5) was treated in 2M ethanolic hydrogen chloride for 6 hours at 80° cholesta-3,5-diene (IV) was formed in 41% yield; no cholesterol was obtained during this experiment showing

[4] M. S. Patel and W. J. Peal, *Tetrahedron*. **20**, 2511 (1964).
[5] C. W. Shoppee, *J. Chem. Soc.* 1147 (1946).

that the 3,5-diene was not obtained by prior hydrolysis to cholesterol followed by dehydration of the sterol.

DISCUSSION

Classical carbonium ion theory[6] predicts that the carbonium ion I would be expected to be the intermediate in both the acid-catalysed dehydration of cholesterol and also during rearrangement, under acidic conditions, of a 3,5-cyclosteroid such as 6β-methoxy-3α,5-cyclo-5α-cholestane (III). However the experimental results described in this paper, together with more recent views[7] on the importance of the leaving group in modifying the structure and reactivity of carbonium ions, necessitate a re-interpretation of the classical carbonium ion concept for these reactions. During the acid-catalysed reactions of cholesterol the leaving group from C_3 is H_2O, whilst, on the other hand, there is no analogous leaving group during the rearrangement of 3,5-cyclosteroids. Since, in this rearrangement, stereochemically pure substitution products are formed rapidly and quantitatively the virtually free, though stereo-specific, carbonium ion I must be the intermediate. The slowness of the acid-catalysed reactions of cholesterol implies that dissociation of the protonated sterol to give H_2O and carbonium ion I is slow. Furthermore, the dominant formation of cholesta-3,5-diene (IV) suggests that other factors are involved.

Cholesterol when treated with hydrogen chloride in all the solvents of Table 1 yielded varying amounts of 3β-substituted steroids. The absence of the 3α-epimers from all reaction products showed that no bimolecular substitution occurred. The formation of 3β-chlorocholest-5-ene (II, R = Cl) also indicates that a carbonium ion of structure similar to I was formed such that the usual stereo-specific products from carbonium ion I were obtained. With alcohol as solvent, some of the corresponding 3β-alkoxycholest-5-ene was usually obtained. Again, this must have been formed through an ion of essentially similar structure to I, by a unimolecular process, since cholestanol did not react significantly with hydrogen chloride in ethanol or 2-chloroethanol.

Cholesterol does not possess the necessary anti-periplanar configuration at C_3-C_4 for bimolecular elimination. Such a configuration is present in epicholesterol which dehydrates readily with ethanolic hydrogen chloride.[8] Furthermore the saturated 3α-hydroxy steroids, sarsasapogenin and smilagenin, are dehydrated under these conditions[9] and it has been shown, in the course of this work, that coprostanol is dehydrated with ethanolic HCl under conditions which leave cholestanol unaffected. However, Shoppee[10] found that epicholesteryl derivatives undergo unimolecular elimination to give cholesta-3,5-diene demonstrating the preference of the epicholesteryl cation to react by proton expulsion from C_4 rather than by reaction with a nucleophile. Similarly[11] the coprostanyl cation tends to give elimination rather than substitution products. The readier acid-catalysed dehydration of 3α-hydroxysteroids may then be explained in two ways: (i) Elimination proceeds by a bimolecular

[6] e.g. [a]C. K. Ingold, *Structure and Mechanism in Organic Chemistry* p. 391, Bell, London (1953); [b]E. D. Hughes, C. K. Ingold and A. M. M. Mandour, *J. Chem. Soc.* 2090, (1948).

[7] D. J. Cram and M. R. V. Sahyun, *J. Amer. Chem. Soc.* **85,** 1257 (1963).

[8] R. E. Marker, O. Kamm, T. S. Oakwood and J. F. Laucius, *J. Amer. Chem. Soc.* **58,** 1948 (1936).

[9] M. E. Wall, S. Serota and L. P. Witnauer, *J. Amer. Chem. Soc.* **77,** 3086 (1955).

[10] C. W. Shoppee and D. F. Williams, *J. Chem. Soc.* 686 (1955).

[11] R. J. Bridgewater and C. W. Shoppee, *J. Chem. Soc.* 1709 (1953).

Table 1. Yields of products formed in the reaction between hydrogen chloride and cholesterol in various solvents at 80°

Solvent	Acid concentration initial	Acid concentration final	1-Hr. heating period: Total[a] reaction %	1-Hr. heating period: Elimination product Cholesta-3,5-diene %	1-Hr. heating period: Substitution products Chloride %	1-Hr. heating period: Substitution products Ether %	6-Hr. heating period: Total[a] reaction %	6-Hr. heating period: Elimination product Cholesta-3,5-diene %	6-Hr. heating period: Substitution products Chloride[b] %	6-Hr. heating period: Substitution products Ether %	6-Hr. heating period: Uncharacterized products[g] A %	6-Hr. heating period: Uncharacterized products[g] B %
1. Di-isopropyl ether	2M	1·900M	—	—	—	—	3·5	0·6	2·2[c]	—	0·7	0·0
2. Dimethyl-formamide	2M	1·850M	—	—	—	—	4·9	1·3	2·9	—	0·7	0·0
3. Dioxan[b]	2M	1·960M	—	—	—	—	11·0	1·6	3·7	—	1·4	4·2
4. Methanol	2M	1·066M	—	—	—	—	25·8	17·1	4·4	2·5	0·7	1·1
5a. Ethanol	2M	1·375M	—	—	—	—	40·7	29·7	7·4	2·5	0·0	1·1
5b. Ethanol	2M	—[e]	9·1	6·4	—[e]	—[e]	—	—	—	—	—	—
5c. Ethanol	1M	—[e]	—	—	—	—	13·4	9·6	—[e]	—[e]	—[e]	—[e]
5d. Ethanol	0·5M	—[e]	—	—	—	—	7·8	3·8	—[e]	—[e]	—[e]	—[e]
6. Propan-1-ol	2M	1·431M	—	—	—	—	51·9	32·8	13·5	0·9	4·5	0·2
7. Propan-2-ol	2M	1·645M	—	—	—	—	36·8	29·1	3·8	2·1	1·8	0·0
8. Butan-1-ol[b]	2M	1·635M	—	—	—	—	51·2	33·1	9·5	1·9	4·2	2·5
9a. 2-Ethoxy ethanol	2M	1·667M	—	—	—	—	84·5	25·9	18·8	—[f]	18·8	20·8[f]

9b. 2-Ethoxy-ethanol	2M	—[e]	33·8	13·2	—[e]	—	—	—	—	—	—	—
10. 3-Chloro-propanol[b]	0·5M	0·290M	29·2	9·4	—[e]	—	—	—	—	—	—	—
11a. 2-Chloro-ethanol[b]	0·5M	—[e]	100	0·0	—[e]	—	—	—	—	—	—	—
11b. 2-Chloro-ethanol[b]	0·067M	—[e]	50·5	11·8	19[d]	ca. 10	—	—	—	—	—	—
11c. 2-Chloro-ethanol[b]	0·0025M	—[e]	—	—	—	—	31·8	9·1	ca. 5	ca. 15	—	2·0

[a] Total reaction—Overall recoveries were between 90–100% in all experiments. "Total reaction" denotes the % yields of recovered products in the total recovered material. Uncharacterised products were assumed to have the same empirical formulae as cholesterol.

[b] Separate experiments in which cholesta-3,5-diene was treated with hydrogen chloride in the various solvents showed that in some cases there was relatively little reaction. On the other hand 2M dioxan HCl and 0·035M HCl in 2-chloroethanol caused extensive changes. Determination of the U.V. spectra of the products of these reactions indicated considerable loss, presumably by polymerisation of the 3,5-diene function since analysis for chlorine showed that only a small part of the changes resulted from direct addition to the 3,5-diene system.

[c] The product was solvolysed with potassium acetate in propionic acid, hydrolysed by methanolic potassium hydroxide to give cholesterol. By this procedure the yield of 3β-chlorocholest-5-ene in the product was indicated.

[d] Yield obtained by solvolysis as in (c) above.

[e] Not estimated.

[f] Includes some ether—indicated from infrared spectrum of gummy products.

[g] Uncharacterised product A represents substances in the cholesta-3,5-diene – 3β-chlorocholest-5-ene fraction whilst product B was a yellow gum and was normally eluted with benzene.

mechanism in 3α-hydroxysteroids since these steroids possess the necessary anti-periplanar configuration at C_3—C_4. This process cannot occur in 3β-hydroxysteroids unless flip of ring A takes place. (ii) The intermediate carbonium ions from 3α-hydroxysteroids are formed more readily than the corresponding ions from the 3β-hydroxy compounds. This explanation is advanced in the following paper[4] for the greater reactivity of the tertiary alcohol 3β-methylcholesterol (3α-hydroxy) over its epimer 3α-methylcholesterol under acid-catalysed conditions. However, if this is the correct mechanistic interpretation, solvent must stabilize protonated 3β-hydroxy steroid (e.g. cholesterol) more than protonated 3α-hydroxysteroid (e.g. epicholesterol) since in unimolecular solvolytic reactions[10] cholesteryl derivatives react faster than the corresponding epicholesteryl compounds.

Since both the usual unimolecular and bimolecular mechanisms of elimination have been excluded for the acid-catalysed dehydration of cholesterol alternative modified processes must be considered. Three possible mechanisms may be envisaged: (a) rearrangement of the C_5—C_6 double bond to C_4—C_5 followed by allylic elimination*, (b) a modified unimolecular process and (c) flip of ring A under the reaction conditions to yield a molecule with the more readily dehydrated 3α-configuration.

It has been found that 3β-chlorocholest-5-ene was virtually unchanged after reaction in 2M ethanolic hydrogen chloride for 6 hours at 80°. Admittedly, the protonation of cholesterol might enhance the possibility of bond migration from C_5—C_6 to C_4—C_5 followed by allylic acid-catalysed elimination. However further evidence against the rearrangement mechanism (a) is discussed in the following paper.[4]

Unimolecular acid-catalysed dehydration mechanisms[12] postulating, at some stage, a free carbonium ion are clearly excluded in the case of cholesterol. Manassen and Klein[13] suggested a modified unimolecular procedure (b), possibly applicable to cholesterol, to explain their results on the dehydration of butan-2-ol. They envisage the positive centre shielded by solvent molecule followed by abstraction of the C_β proton by this shielding molecule.

Since the 3α-hydroxy configuration is favourable for acid-catalysed elimination reactions it is possible that cholesterol, or the protonated sterol, undergoes flip of ring A (mechanism c) to give this configuration. Elimination of water may then occur by either of the two processes suggested for the dehydration of 3α-hydroxysteroids.

The three mechanisms postulated for the acid-catalysed dehydration of cholesterol have one factor in common, namely, that solvent stabilizes the protonated sterol preventing formation of carbonium ion I. However the borderline nature of reactions in secondary systems and the complexities introduced with varying solvents[14] emphasize the caution with which reactions such as these must be interpreted.

* W. R. Nes and J. A. Steele [*J. Org. Chem.* **22**, 1457 (1957)] found that dehydration of ergosterol did not involve a free carbonium ion and suggested this rearrangement mechanism. However more recent work by K. Tsuda, R. Hayatsu, J. A. Steele, O. Tanaka and E. Mosettig, *J. Amer. Chem. Soc.* **85**, 1126 (1963) makes doubtful the original interpretation of this reaction.

[12a] D. S. Noyce and C. A. Lane, *J. Amer. Chem. Soc.* **84**, 1635 (1962);
[b] J. Rocek, *Coll. Czech. Communs.* **25**, 375 (1960);
[c] R. H. Boyd, R. W. Taft, A. P. Wolf and D. R. Christman, *J. Amer. Chem. Soc.* **82**, 4729 (1960).
[13] J. Manassen and F. S. Klein, *J. Chem. Soc.* 4203 (1960).
[14a] C. A. Bunton and R. B. Henderson, *Tetrahedron Letters* 1829 (1963);
[b] H. Weiner and R. A. Sneen, *Ibid.* 1309 (1963).

The solvents used in the reactions (Table 1) may be divided into three groups:

(A) Those in which reaction occurs slowly giving dominantly substitution products, e.g. di-isopropyl ether, dimethylformamide and dioxan.

(B) Those in which reaction occurs more rapidly giving dominantly elimination products, e.g. methanol, ethanol, propan-1-ol, propan-2-ol, butan-1-ol, 2-ethoxyethanol.

(C) Those in which reaction occurs very rapidly with the dominant production under conditions of low acidity of substitution products e.g. 3-chloropropanol, 2-chloroethanol.

Dioxan is intermediate between groups A and B. However, experimental uncertainties with this solvent are rather large, owing to the considerable reactivity of cholesta-3,5-diene towards 2M hydrogen chloride in dioxan. 2-Ethoxyethanol similarly shows properties between those of Group B and C.

The slowness of the reactions in solvents of the first group is probably accounted for by the low dissociation of HCl in the solvent.[15,16] The dominant substitution reaction is presumably due to the inability to stabilize the protonated sterol, thus leading to the production of a carbonium ion of similar structure to I and the formation of substitution products.

The differing reactivities in solvents in categories B and C, however, cannot be explained by variations in acid strength or dielectric constant. In Group B reactivity is least in methanol yet hydrogen chloride is highly dissociated[15] in this solvent which has also the highest dielectric constant[17] of all the alcohols cited in the Table. This low reactivity may be partly explained by greater acid loss than in other alcohols. The results suggest that the differences in reactivity lie in differences in solvation of the protonated species in the various solvents, i.e. it is possible that in class B solvents only a small proportion of protonated sterol molecules pass to a carbonium ion of type I yielding substitution products, the major reaction course (elimination) taking place from the solvent-stabilised protonated cholesterol.

The high reactivity of hydrogen chloride in 2-chlorethanol towards cholesterol is unexpected. It is noteworthy that substitution is the dominant reaction with this solvent in very dilute acid concentrations. The ready formation of the carbonium ion I in this solvent indicates that solvation is reduced. When reaction was carried out in 2-chloropropanol a reactivity intermediate between those of hydrogen chloride in propanol and in 2-chloroethanol was observed.

EXPERIMENTAL

Light petroleum had b.p. 60–90°. $[\alpha]_D$ are for $CHCl_3$ solutions at 25°. UV spectra were determined on a Unicam S.P. 500 in cyclohexane and IR spectra on a Perkin–Elmer Infracord spectrophotometer model 137. A general procedure was adopted for the isolation of the products of the reaction between cholesterol or its derivatives and hydrogen chloride in various solvents. After completion of the reaction the solution was chilled, poured into excess saturated Na_2CO_3 aq and partially concentrated under red. press. to remove the solvent. The residue was extracted with ether and the ether solution washed with water, dried ($MgSO_4$) and chromatographed on alumina from a light petroleum solution.

[15] C. J. Janz and S. S. Danyluk, *Chem. Rev.* **60**, 209 (1960).

[16a] W. Gerrard and E. D. Macklen, *Chem. Rev.* **59**, 1105 (1959);

[b] A. J. Parker, *Quart. Rev.* **16**, 163 (1962).

[17] A. Weissberger, E. S. Proskauer, J. A. Riddick and E. E. Toops, *Techniques of Organic Chemistry* VII *Organic Solvents* (2nd Edition) p. 270. Interscience, New York (1955).

Reaction between 2M *ethanolic hydrogen chloride and* 6β-*methoxy*-3α,5-*cyclo*-5α-*cholestane* (III)

Hydrogen chloride in dry ethanol (55 ml, 3·6M) was added to a refluxing solution of the 3,5-cyclosteroid (510 mg) in dry ethanol (45 ml). After heating for a further 3 min the products were isolated by the general procedure. Chromatography of the product on alumina from light petroleum gave initially 3β-chlorocholest-5-ene (300 mg), m.p. and mixed m.p. with an authentic sample 96–97°. No peak at 236 mμ was noted in the UV spectrum indicating the absence of cholesta-3,5-diene. Further elution with light petroleum gave 3β-ethoxycholest-5-ene (198 mg), m.p. and mixed m.p. with an authentic specimen 88–89°. No further substances were obtained from the column.

Cholesta-3,5-*diene* (IV). A commercial sample had m.p. 77·5–79·5°, $[\alpha]_D$ −107° (*c*, 0·5), ϵ_{236}, 20,400; after recrystallization from methanol–ethyl acetate it had m.p. 79·5–81°, $[\alpha]_D$ −117° (*c*, 0·97), ϵ_{236}, 21,100.

During the elimination reactions with 2M HCl in various solvents several specimens of high purity were isolated, e.g. 2M HCl in propan-2-ol gave cholesta-3,5-diene, m.p. 78·5–79·5°, $[\alpha]_D$ −8° (*c* 0·93), ϵ_{236}, 21,800.

In the review in Elsevier's encyclopaedia[18] on criteria of purity of cholesta-3,5-diene three categories are defined, (1) pure cholesta-3,5-diene ($[\alpha]_D$ from −110° to −129°), (2) laevorotatory solid cholesterilene ($[\alpha]_D$ from −50° to −110°) and (3) dextrorotatory liquid cholesterilene ($[\alpha]_D$ from 0 to +41°). All specimens obtained in the course of this work come within the category of 'laevorotatory solid cholesterilene'. The highest recorded values[18,19] of ϵ_{235} are 21,500 and 22,000 (EtOH) for cholesta-3,5-diene. Since these are similar to the highest values obtained in this work, the value ϵ_{236} = 22,000 (cyclohexane) is used in all calculations of 3,5-diene yield. The cause of the considerable $[\alpha]_D$ variations remains obscure. It was noted that when a gummy reaction product containing 3,5-diene was left to stand a change in ϵ_{236} and $[\alpha]_D$ occurred; thus a sample which had, on isolation, $[\alpha]_D$ −67°, ϵ_{236}, 16,600 had changed after 3 days to $[\alpha]_D$ −8·6° and ϵ_{236}, 8,700 and was much less soluble in organic solvents. Some of the polymeric products, obtained from cholesterol and cholest-3,5-diene on strong acid or other treatment, are dextrorotatory.[20]

3β-*Chlorocholest*-5-*ene* (cholesteryl chloride; II, R = Cl). No pure samples of the chloride were isolated from any reaction product. However, the characteristic IR absorption spectrum was noted in the products of all reactions between cholesterol and HCl in various solvents. Confirmation of the presence of the chloride was carried out by solvolysis of the chloride similar to the method of Shoppee.[5] In a trial experiment pure 3β-chlorocholest-5-ene (216 mg) was refluxed for 6 hr in propionic acid (10 ml) containing potassium acetate (300 mg). The product was isolated and hydrolysed with 3% methanolic KOH (30 ml), under reflux for 1 hr. The residue obtained on isolation of the product in the usual way was chromatographed on alumina from light petroleum. Elution with benzene–chloroform (1:1) gave cholesterol (146 mg) m.p. and mixed m.p. 148–149 after recrystallization.

3β-*Alkoxycholest*-5-*enes* (II, R = OAlk). During the reaction between 2M HCl and alcohols the corresponding ethers were almost invariably obtained. These usually separated satisfactorily during the chromatographic analysis of the products. They were identified by m.p., $[\alpha]_D$, IR spectrum and, in most cases, mixed m.p. with authentic specimens.

3β-(2-*Chloroethoxy*)-*cholest*-5-*ene* (II, R = OCH_2CH_2Cl). Cholesteryl toluene-*p*-sulphonate (450 mg) was dissolved in 2-chloroethanol (5 ml) by gentle warming on the water bath. Methanol (5 ml) was added and the solution left to crystallize overnight. The product (200 mg) had, after 3 recrystallizations from methanol–ethyl acetate, m.p. 89–90°, $[\alpha]_D$ −29° (*c* 0·9). (Found: C, 77·6; H, 10·6; Cl, 8·0. $C_{29}H_{49}OCl$ requires: C, 77·7; H, 11·0; Cl, 7·9%).

Reaction between 2M *ethanolic hydrogen chloride and cholesterol in various solvents at* 80°

Methanol, ethanol, propan-1-ol, propan-2-ol, butan-1-ol, 2-ethoxyethanol, dioxan and di-isopropyl ether were purified by the usual procedures.[21]

Dimethylformamide (b.p. 146–146·5°/660 mm) was fractionated from anhydrous K_2CO_3 after standing overnight with this reagent.

[18] '*Elseviers' Encyclopaedia of Organic Chemistry* Series III **14**, pp. 1411S–1415S. Elsevier, Amsterdam (1954).

[19] W. G. Dauben and F. G. Willey, *Tetrahedron Letters* 893 (1962).

[20] Ref. 18, pp. 1415S–1417S, but see also pp. 3645S–3650S.

[21] Ref. 17, pp. 333 *et seq.*

2-Chloroethanol (b.p. 123·5–124°/660 mm) was dried over a mixture of anhydrous $MgSO_4$ and anhydrous Na_2CO_3 and doubly fractionated. An acidity check showed the purified 2-chloroethanol to be 0·0012M.

3-Chloropropanol (b.p. 78°/25 mm) was purified in a similar manner. The acidity of the purified product was found to be 0·0026M.

The purity of the solvents was checked by gas chromatography.

Dry hydrogen chloride gas was passed into the pure solvent until the acid strength was approximately 4M. The acidity was determined titrimetrically.

Cholesterol was purified through the 5:6-dibromo derivative. After two recrystallizations the purified material had m.p. 149·5° and $[\alpha]_D$ −39°.[22]

Cholesterol (usually 1 g) in an ampoule, was dissolved in a volume of the solvent such that on addition of the required amount of HCl:solvent the total volume gave a solution of 0·026M in cholesterol (100 ml with 1 g cholesterol) and 2M in HCl. The ampoule was at once sealed and placed in a thermostat at 80° ± 0·5° for the required time. After the reaction the ampoule was chilled, opened and the products worked up as described. Unchanged cholesterol was recovered, in almost all the experiments, on elution of the alumina chromatograms with benzene–chloroform mixtures. It was identified from (1) IR spectrum, (2) m.p. and, on occasion, mixed m.p.

A sample of 2M HCl in each of the various solvents was treated in a sealed tube for 6 hr at 80°. The acid strength at the end of the heating period was determined titrimetrically in order to estimate the acid lost by reaction with solvent (Table 1).

(1) *Di-isopropyl ether.* Cholesterol (500 mg) treated in 2M HCl–di-isopropyl ether (50 ml) at 80° for 6 hr gave, after the usual isolation procedure, 477 mg of recovered material. This yielded on elution of the chromatogram with light petroleum, product (17 mg) with ϵ_{236}, 3,300 showing cholesta-3,5-diene (2·5 mg, 0·6%) and Cl, 4·65% showing 3β-chlorocholest-5-ene (9 mg, 1·8%). Cholesterol (477 mg) was eluted with chloroform. Confirmation of the presence of the chloride was obtained from an IR spectrum and by solvolysis of a second sample of product obtained in an analogous experiment. Product (97 mg) gave cholesterol (41 mg) identified from m.p., mixed m.p. 147–148° and an IR spectrum. Solvolysis thus indicated a yield of 2·2% 3β-chlorocholest-5-ene.

(2) *Dimethylformamide.* Cholesterol (1 g) treated in 2M HCl–dimethylformamide (100 ml) at 80° for 6 hr gave on isolation and chromatography unchanged cholesterol (922 mg) and product (47 mg). The product had ϵ_{236}, 5,600 showing cholesta-3,5-diene (12·0 mg, 1·3%) and Cl, 5·39% showing 3β-chlorocholest-5-ene (29 mg, 2·9%) confirmed from an IR spectrum.

(3) *Dioxan.* Cholesterol (1 g) when heated in 2M HCl–dioxan (100 ml) for 6 hr at 80° gave after isolation and chromatography cholesterol (880 mg) and total product (109 mg). Elution with light petroleum of the alumina chromatogram gave a fraction (67 mg), ϵ_{236}, 4,900; Cl, 4·97%, indicating cholesta-3,5-diene (15 mg, 1·6%) and 3β-chlorocholest-5-ene (38 mg, 3·7%). Elution with benzene gave an uncharacterized yellow gum (42 mg, 4·2%) from which crystals, m.p. 220–225°, were obtained on crystallization from methanol–ethyl acetate.

(4) *Methanol.* Cholesterol (1 g) on heating in 2M HCl–methanol for 6 hr at 80° gave, after the usual isolation procedure, 948 mg of recovered material. Elution with light petroleum during analysis by alumina chromatography gave a fraction (206 mg), ϵ_{236}, 16,500 indicating cholesta-3,5-diene (155 mg, 17·1%) and Cl, 1·86% indicating 3β-chlorocholest-5-ene (44 mg, 4·4%). The presence of the chloride was confirmed since on solvolysis cholesterol (14 mg), identified by m.p. mixed m.p. 147–148° and an IR spectrum, was obtained from this fraction (175 mg). Further elution with light petroleum gave 3β-methoxycholest-5-ene (25 mg, 2·5%), m.p. and mixed m.p. with an authentic specimen 78–80°. Elution with benzene yielded an uncharacterized yellow gum (11 mg, 1·1%). Unchanged cholesterol (710 mg) was eluted with benzene–chloroform mixtures.

(5a) *Ethanol.* Cholesterol (1 g) on treatment in 2M HCl–ethanol (100 ml) at 80° for 6 hr gave, after isolation and chromatography, total product (375 mg) and unchanged cholesterol (559 mg). Chromatographic analysis of the product on alumina gave, on elution with light petroleum, a fraction (251 mg), ϵ_{236}, 18,000; Cl, 1·57%. Further elution with light petroleum gave a fraction (87·5 mg), ϵ_{236}, 15,100; Cl, 2·75%; yields of cholesta-3,5-diene (266 mg, 29·7%) and 3β-chlorocholest-5-ene (72 mg, 7·4%). Elution with benzene–light petroleum (1:3) gave 3β-ethoxycholest-5-ene (25·5 mg, 2·5%), m.p. and mixed m.p. with an authentic specimen 88·5–90°. Elution with benzene yielded an uncharacterized yellow gum (11 mg, 1·1%).

[22] Ref. 2, p. 28.

(5b) *Ethanol.* Cholesterol (200 mg) on treatment for 1 hr at 80° in 2M HCl–ethanol (20 ml) gave, after isolation and chromatography, unchanged cholesterol (180 mg) and product (18 mg). Cholesta-3,5-diene (12 mg, 6·4%) was shown to be present from the ϵ_{236}, 14,200 of the product.

(5c) *Ethanol.* Cholesterol (200 mg) on treatment for 6 hr at 80° in 1M HCl–ethanol gave, after isolation and chromatography, unchanged cholesterol (162 mg) and product (24 mg). Cholesta-3,5-diene (17 mg, 9·6%) was indicated from the ϵ_{236}, 15,800 of the product.

(5d) *Ethanol.* Cholesterol (200 mg) on heating for 6 hr in 0·5M HCl–ethanol gave, after isolation and chromatography, product (14 mg) and unchanged cholesterol (180 mg). Cholesta-3,5-diene (7 mg, 3·8%) was estimated from the ϵ_{236}, 11,300 of the product.

(6) *Propan-1-ol.* Cholesterol (1 g) when heated in 2M HCl–propan-1-ol (100 ml) for 6 hr at 80° gave, after isolation and chromatography, unchanged cholesterol (470 mg) and product (482 mg). Elution of the alumina column with light petroleum gave a fraction (484 mg) containing cholesta-3,5-diene (304 mg, 32·8%) and 3β-chlorocholest-5-ene (136 mg, 13·5%), indicated by ϵ_{236}, 13,800 and Cl, 2·46% respectively. Further elution gave successively 3β-propoxycholest-5-ene (9 mg, 0·9%) identified from an IR spectrum, uncharacterized yellow gum (2 mg, 0·2%) and cholesterol (470 mg) under similar eluant conditions to those of previous experiments.

(7) *Propan-2-ol.* Cholesterol (1 g) when treated with 2M HCl–propan-2-ol (100 ml) for 6 hr at 80° gave, after isolation and chromatography, unchanged cholesterol (603 mg) and total product (370 mg). Elution of the alumina chromatogram with light petroleum gave an initial fraction (304 mg), ϵ_{236}, 19,900; Cl, 0·0%, further elution with light petroleum gave a product (43 mg), ϵ_{236}, 500; Cl, 7·94%; total yields cholesta-3,5-diene (276 mg, 29·1%) and 3β-chlorocholest-5-ene (39 mg, 3·8%). Elution with light petroleum–benzene (3:1) gave 3β-isopropoxycholest-5-ene (23 mg, 2·1%) identified from m.p. and mixed m.p. 130–132° and an IR spectrum. Further elution gave only unchanged cholesterol (603 mg).

After recrystallization from methanol–ethyl acetate the first fraction from this chromatographic analysis had m.p. 78·5–79·5°, $[\alpha]_D$ −89° and ϵ_{236}, 21,800.

(8) *Butanol.* Cholesterol (1 g), when heated in 2M HCl–butanol (100 ml) yielded, on isolation and chromatographic analysis, product (496 mg) and unchanged cholesterol (482 mg). Elution of the alumina chromatogram with light petroleum gave a fraction (339 mg), ϵ_{236}, 17,400; Cl, 0·98% and further elution with the same solvent gave a fraction (110 mg), ϵ_{236}, 8,600; Cl, 3·29%, indicating yields of cholesta-3,5-diene (311 mg, 33·1%) and 3β-chlorocholest-5-ene (97 mg, 9·5%). Successive elution using the same solvent systems as before yielded 3β-butoxycholest-5-ene (22 mg, 1·9%) identified from its IR spectrum, uncharacterized yellow gum (25 mg, 2·5%) and cholesterol.

(9a) *2-Ethoxyethanol.* Cholesterol (200 mg) when heated in 2M HCl–2-ethoxyethanol (20 ml) for 6 hr at 80° gave, on isolation and chromatography, product (165 mg) and unchanged cholesterol (31 mg). Chromatographic analysis of the product yielded, on elution with light petroleum, a fraction (124 mg), ϵ_{236}, 8,700; Cl, 2·69% giving cholesta-3,5-diene (49 mg, 25·9%) and 3β-chlorocholest-5-ene (38 mg, 18·8%). Further elution of the column gave a gum (41 mg) with an IR spectrum showing it to be very crude 3β-(2-ethoxyethoxy)-cholest-5-ene.

(9b) *2-Ethoxyethanol.* Cholesterol (200 mg) when heated at 80° for 1 hr in 2M HCl–2-ethoxyethanol (20 ml) gave, on isolation and chromatographic analysis, unchanged cholesterol (123 mg) together with product (62 mg). Elution with light petroleum of the alumina column gave a fraction (47·4 mg), ϵ_{236}, 10,800 indicating cholesta-3,5-diene (23 mg, 13·2%). Further elution gave gums (15 mg) which showed similar spectra to that of 3β-(2-ethoxyethoxy)-cholest-5-ene.

(10) *3-Chloropropanol.* Cholesterol (100 mg) when heated for 1 hr in 0·5M HCl–3-chloropropanol (5 ml) gave product (28 mg) and unchanged cholesterol (68 mg). Cholesta-3,5-diene (9 mg, 9·4%) was shown to be present since the product had ϵ_{236}, 7,500.

(11a) *2-Chloroethanol.* Cholesterol (200 mg) when heated for 1 hr at 80° with 0·5M HCl–2-chloroethanol (50 ml) gave, on isolation and chromatography, product (198 mg). Alumina chromatography of the product yielded an initial fraction (165 mg) on elution with light petroleum. This fraction had no peak at 236 mμ—its IR spectrum showed the presence of some 3β-chlorocholest-5-ene. Further successive elution of the column gave uncharacterized gums (34 mg) with benzene and benzene–chloroform mixtures.

(11b) *2-Chloroethanol.* Cholesterol (500 mg) on heating at 80° for 1 hr in 2-chloroethanol containing HCl (0·067M) gave product (251 mg) and unchanged cholesterol (245 mg). Elution of the alumina chromatogram with light petroleum yielded a fraction (228 mg), ϵ_{236}, 5,400 of which

cholesta-3,5-diene (56 mg, 11·8%) formed part. Solvolysis of a sample of this fraction (220 mg) by propionic acid–potassium acetate yielded, on hydrolysis, isolation and chromatography, cholesterol (67 mg) indicating the presence of 3β-chlorocholest-5-ene (99 mg, 19%). The IR spectrum of this fraction showed 3β-(2-chloroethoxy)-cholest-5-ene (ca. 10% by comparison with spectra of mixtures of the 3 components).

(11c) 2-*Chloroethanol.* Cholesterol (200 mg) on heating for 6 hr at 80° in 0·0025M HCl–2-chloroethanol gave, after isolation and chromatographic analysis, product (62 mg) and unchanged cholesterol (113 mg). Elution of the alumina chromatogram with light petroleum gave a fraction (54 mg), ϵ_{236}, 6,800 showing cholesta-3,5-diene (17 mg, 9·1%) and, by comparison of the IR spectra with those of mixtures of the main components, ca. 15% 3β-(2-chloroethoxy)-cholest-5-ene and ca. 5% 3β-chlorocholest-5-ene. Further successive elution of the column gave, with light petroleum, 3β-(2-chloroethoxy)-cholest-5-ene (4 mg), m.p. and mixed m.p. with an authentic specimen 87–88° and, with benzene, a yellow uncharacterized gum (4·5 mg) and with chloroform, cholesterol.

Reaction between cholesterol and 2M *ethanolic hydrogen chloride at room temperature*

Cholesterol (100 mg) was dissolved in 2M ethanolic HCl (25 ml) and left for 182 days at laboratory temp in the dark. The product (88 mg), after isolation in the usual manner, was chromatographed from light petroleum on alumina giving, with this solvent, crude cholesta-3,5-diene (22 mg), ϵ_{236}, 17,300; $[\alpha]_D$ −72° (*c* 0·9) which after recrystallization had m.p. 77–79°. Cholesterol (66 mg) was eluted with chloroform.

Reaction between cholesterol and 0·5M 2-*chloroethanolic hydrogen chloride at room temperature*

Cholesterol (212 mg) was left in 0·5M 2-chloroethanolic HCl (25 ml) for 4 days. Reaction product (84 mg) and unchanged cholesterol (100 mg) were isolated and separated in the usual way. The fraction (60 mg) eluted with light petroleum had ϵ_{236}, 4,900 showing cholesta-3,5-diene (13 mg, 7·5%) and, when solvolysed, gave cholesterol (20 mg) indicating the presence of 3β-chlorocholest-5-ene (30 mg, 16%). Elution of the column with benzene yielded uncharacterized gum (24 mg) whilst chloroform gave cholesterol.

Reaction between 3β-*ethoxycholest-5-ene* (II, R = OC_2H_5) *and* 2M *hydrogen chloride in ethanol*

The ether (100 mg) was heated at 80° for 6 hr in 2M HCl–ethanol (20 ml). Elution with light petroleum of the alumina chromatogram, prepared after the products had been worked up in the usual way, gave a fraction (24 mg), ϵ_{236}, 17,100; Cl, 0·0%. Further elution with the same solvent yielded a fraction (71 mg), ϵ_{236}, 6,200; Cl, 0·0%. The IR spectra of both these samples showed that starting material 3β-ethoxycholest-5-ene was present. The total yield of cholesta-3,5-diene was 39 mg (43%). Elution of the column with benzene gave a yellow gum (1 mg). No eluates were obtained with chloroform.

*Reactions between cholesta-*3,5-*diene* (IV) *and hydrogen chloride in various solvents*

Ethanol. Cholesta-3,5-diene (100 mg), ϵ_{236}, 21,500 was heated for 6 hr at 80° in 2M ethanolic HCl (20 ml) and the products (99 mg) isolated in the usual way. Elution of the alumina chromatogram with light petroleum gave a gum (95 mg), ϵ_{236}, 15,700 and Cl, 0·0%. Further elution with benzene gave an uncharacterized yellow gum (4 mg). When in a similar experiment heating was stopped after 4 hr the total product had ϵ_{236}, 18,000.

Butanol. Cholesta-3,5-diene (100 mg) was heated for 6 hr at 80° in 2M butanolic HCl. After the usual isolation procedure the gummy product (83 mg) had ϵ_{236}, 12,800; Cl, 0·0%.

2-*Chloroethanol.* Cholesta-3,5-diene (50 mg) was heated for 1 hr at 80° in 0·035M HCl–2-chloroethanol (20 ml). The yellow gummy product (50 mg) was isolated in the usual way and had ϵ_{236}, 11,100; Cl, 0·81%.

Dioxan. Cholesta-3,5-diene (100 mg) when heated in 2M HCl–dioxan for 6 hr gave a crude product (99 mg) after the usual isolation procedure. Elution of the alumina chromatogram with light petroleum yielded a fraction (63 mg), ϵ_{236}, 12,300 and Cl, 0·0% indicating unchanged cholesta-3,5-diene (33 mg, 34%). Elution with benzene gave an uncharacterized yellow gum (36 mg), Cl, 0·0%.

No investigation of the products of these reactions has been made beyond noting the extent of reaction between cholesta-3,5-diene and HCl in the various solvents. In Table 1 the uncharacterized products A and B are considered to arise largely, if not entirely, from secondary reaction between cholesta-3,5-diene and HCl. In Table 1 those solvents in which the yields of A and B are greatest are those solvents in which, in the above experiments, cholesta-3,5-diene reacts to the greatest extent.

Reaction between 3β-chlorocholest-5-ene (II, R = Cl) *and hydrogen chloride in ethanol*

The chloride (100 mg), ϵ_{236}, 100 when heated in 2M ethanolic HCl for 6 hr at 80° gave a solid product (87 mg), ϵ_{236}, 700 (cholesta-3,5-diene, 3%); $[\alpha]_D$ −25° (*c* 0·87), IR spectrum that of 3β-chlorocholest-5-ene.

Reaction between cholestanol and hydrogen chloride in ethanol

Cholestanol[23] (100 mg), m.p. 142–3°; $[\alpha]_D$ +25° was heated for 4 days at 80° in 2M ethanolic HCl (20 ml). Chromatography of the product after the usual isolation procedure gave, on elution with light petroleum, a fraction (2·5 mg). Further elution of the column with benzene–chloroform yielded unchanged cholestanol.

Action of ethanolic hydrogen chloride on coprostanol

Coprostanol[23] (156 mg), m.p. 100–101°; $[\alpha]_D$ +31° (*c* 0·5) was heated for 24 hr in 2M ethanolic HCl. Isolation and chromatography of the product yielded a gum (17 mg) from light petroleum and unchanged coprostanol (113 mg) from benzene. The gum (17 mg) on titration with monoperphthalic acid showed coprostene (9·2 mg = 2·00 ml of 0·025N $Na_2S_2O_3$; 7·4%). In a similar experiment the product (30 mg) eluted with light petroleum had Cl, 1·4% showing 5 mg (1·8%) of a chlorinated coprostane; this compound may have arisen from direct substitution of coprostanol or by addition of HCl to coprostene.

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[23] Ref. 2. p. 34.