944. *Steroids and Walden Inversion. Part XLIV.* The Acetolysis of Cholesteryl Iodide.*

By C. W. Shoppee, W. Connick, and R. H. Davies.

Acetolysis of cholesteryl iodide in the presence of added acetate has been found to involve a unimolecular heterolysis, confirming the view [1] that the replacement, accomplished with retention of configuration, involves the 3β-cholesteryl cation. In the absence of added acetate, the reaction is catalysed by hydrogen ions and is reversible. The presence of acetic anhydride inhibits such catalysis, but the extent of inhibition is dependent on the concentration of acetic anhydride and the temperature.

With weakly nucleophilic reagents replacements at $C_{(3)}$ in Δ^5-steroids take place with complete retention of configuration; thus acetolysis of cholesteryl chloride at 100° gives 91% of cholesteryl acetate.[1] Kinetic studies of the acetolysis of cholesteryl toluene-*p*-sulphonate [2] and bromide [3] have confirmed Shoppee's original view that such reactions proceed by unimolecular heterolysis (S_N1).

Acetolysis of cholesteryl bromide was found to present unusual features when compared with that of cholesteryl toluene-*p*-sulphonate. In the absence of added acetate, the former reaction was reversible and catalysed by hydrogen ions although the rate-determining stage was ionisation of the bromide; further, in the presence of acetic anhydride the reaction gave a first-order rate constant which was independent of the acetic anhydride concentration over a wide range. Acetolysis of epicholesteryl bromide [4] also presented similar features when compared with that of epicholesteryl toluene-*p*-sulphonate.

Although it was not expected that acetolysis of cholesteryl iodide would depart from unimolecular (S_N1) characteristics, it was of interest to obtain kinetic data for comparison with those for the toluene-*p*-sulphonate and bromide, and to elucidate any additional features which might arise from the reducing properties of hydrogen iodide formed in the absence of added acetate.

Preliminary rate measurements, carried out at 93·8° by the sealed-ampoule procedure,[3] showed that 0·02M-solutions of cholesteryl iodide in solutions of diphenylguanidinium acetate in acetic acid rapidly darkened, so that location of the end-point in the subsequent titration of the liberated hydriodic acid became very difficult. The colour was due to iodine formed by the action of dissolved oxygen on the hydrogen iodide and diphenylguanidinium iodide in the ampoules; a degassing technique was accordingly devised, and the solutions then remained colourless even after 40 hr. In the absence of diphenylguanidinium acetate, and in the presence of acetic anhydride, solutions of cholesteryl iodide in acetic acid still slowly became yellow.

Tables 1 and 2 show selected results for the first-order integrated rate constants for the acetolysis of 0·02M-cholesteryl iodide, with 0·02M- and 0·05M-diphenylguanidinium acetate in acetic acid at 93·8°, calculated from the equation, $k = (1/t) \ln [a/(a - x)]$, where a is the initial molar concentration of cholesteryl iodide, and x the molar concentration of hydrogen iodide liberated after t min. The experimental limitation of ±0·01 ml. in titration of the liberated hydrogen iodide introduces an uncertainty of about ±5% in the individual values of k for short intervals.

These rate constants agree within experimental accuracy up to 40% reaction. Thus increasing the acetate concentration of the solvent from 0·02M to 0·05M resulted only in an increase in the initial rate constant from $4{\cdot}0 \times 10^{-4}$ to $7{\cdot}0 \times 10^{-4}$ min.$^{-1}$. These

* Part XLIII, *J.*, 1959, 2786.

[1] Shoppee, *J.*, 1946, 1147.
[2] Winstein and Adams, *J. Amer. Chem. Soc.*, 1948, **70**, 838.
[3] Davies, Meecham, and Shoppee, *J.*, 1955, 679.
[4] Shoppee and Williams, *J.*, 1955, 686.

initial rate constants may be compared with the value of $1\cdot6 \times 10^{-4}$ min.$^{-1}$ at 93·8° obtained in the absence of added acetate ions. The results indicate a first-order dependence of the rate constant on the acetate concentration. There are, however, several reasons for believing that the acceleration in rate is due to salt effects on a unimolecular reaction

TABLE 1. *Acetolysis of* 0·02M-*cholesteryl iodide* ($10^5a = 10\cdot15$) *in solutions of* 0·02M-*diphenylguanidinium acetate in acetic acid at* 93·8° ± 0·2°.

Time (min.)	10^5x	$10^2x/a$	10^4k (min.$^{-1}$)	Time (min.)	10^5x	$10^2x/a$	10^4k (min.$^{-1}$)
120	0·48	4·7	3·98	787	2·42	23·8	3·46
215	0·72	7·1	3·41	1080	3·23	31·8	3·54
300	1·07	10·5	3·70	1200	3·60	35·4	3·65
335	1·32	13·0	4·15	1539	4·02	39·6	3·27
434	1·50	14·7	3·67	1620	4·34	42·8	3·44
475	1·63	16·1	3·85	1823	4·39	43·8	3·00
582	1·96	19·3	3·68	2172	5·03	49·5	3·15
700	2·25	22·1	3·57	2365	5·20	51·2	3·04

TABLE 2. *Acetolysis of* 0·02M-*cholesteryl iodide* ($10^5a = 10\cdot15$) *in solutions of* 0·05M-*diphenylguanidinium acetate in acetic acid at* 93·8° ± 0·2°.

Time (min.)	10^5x	$10^2x/a$	10^4k (min.$^{-1}$)	Time (min.)	10^5x	$10^2x/a$	10^4k (min.$^{-1}$)
110	0·69	6·8	6·33	801	4·25	41·8	6·77
209	1·40	13·7	7·09	1006	4·86	47·9	6·48
240	1·67	16·5	7·00	1189	5·39	53·1	6·36
376	2·39	23·5	7·11	1189	5·34	52·6	6·27
420	2·49	24·5	6·69	1337	5·62	55·4	6·02
450	2·70	26·6	6·86	1691	6·74	66·4	6·28
650	3·53	34·7	6·81	2253	7·42	72·9	5·24
755	3·83	38·2	6·39				

rather than to a bimolecular substitution by acetate ions. Thus, the accelerating influence of 0·02M-diphenylguanidinium acetate was found to be smaller than that of 0·02M-diphenylguanidinium perchlorate. Moreover, bimolecular attack by acetate ions would lead to inversion and the formation of epicholesteryl acetate. Cholesteryl acetate, cholesta-3,5-diene, and cholesterol were the only products of acetolysis. The possible formation of epicholesteryl acetate as an intermediate is ruled out since this acetate was found to be stable under the above conditions. The influence of acetate concentration on the rate of acetolysis follows a pattern of salt effects observed for unimolecular reactions in the less polar solvents, *e.g.*, in the presence of 0·01M- and 0·03M-diphenylguanidinium acetate the rate constants for the acetolysis of cholesteryl toluene-*p*-sulphonate at 50° are $19\cdot6 \times 10^{-3}$ and $22\cdot0 \times 10^{-3}$ min.$^{-1}$, respectively, to be compared with the value of $7\cdot80 \times 10^{-3}$ min.$^{-1}$ in the pure solvent.[5] The rate of ionisation of organic substrates in solvents of low polarity such as acetic acid may be considerably increased by the addition of electrolytes as a result of reduction or elimination of ion-pair return.[6] A more detailed study of the influence of added salts on the rate of acetolysis of various steroid derivatives is in progress, and it appears that the rate constant for cholesteryl iodide is more sensitive to the influence of added electrolytes than is that for the corresponding bromide [3] or toluene-*p*-sulphonate.[5]

The unimolecular nature of the reaction is confirmed by the progressive fall of the specific rate constants in the later stages of the reaction as shown in curves III and IV of Fig. 1, in which the broken line curves indicate the course of reaction if constant specific rates are assumed. If the acetolysis involves the unimolecular heterolysis:

$$RI \underset{(2)}{\overset{(1)}{\rightleftharpoons}} R^+ + I^- \xrightarrow[(3)]{OAc^-} ROAc + I^-$$

[5] Winstein and Clippinger, *J. Amer. Chem. Soc.*, 1956, **78**, 2784.
[6] Winstein, Smith, and Darwish, *J. Amer. Chem. Soc.*, 1959, **81**, 5512.

the reversibility of the rate-determining ionisation (1) leads to progressive retardation of the reaction.[7] The diphenylguanidinium acetate neutralises the hydrogen iodide liberated, and the resulting iodide ion concentration leads to gradual increase in the importance of reaction (2) and to a progressive fall in specific rate. This " mass law " effect is further substantiated by the retardation produced by the addition of potassium iodide (curve V, Fig. 1). If the ionic strength of the solvent, 0·02M-diphenylguanidinium acetate with 0·03M-potassium iodide, is assumed to be comparable with that of a 0·05M-diphenylguanidinium acetate solution (curve II, Fig. 2), it will be seen that addition of the common iodide ion results in a decrease of the specific rate constant from $7{\cdot}0 \times 10^{-4}$ to about $2{\cdot}6 \times 10^{-4}$ min.$^{-1}$.

The rate constant observed, $4{\cdot}0 \times 10^{-4}$ min.$^{-1}$, in 0·02M-diphenylguanidinium acetate solution at 93·8° (curve II, Fig. 1), is increased in the added presence of 0·5M-water (curve

FIG. 1. *Acetolysis of* 0·02M-*cholesteryl iodide in* 0·02M-*solutions of diphenylguanidinium acetate in acetic acid at* (I) 83·8°, (II) 93·8°, (III) 103·8°, *and* (IV) 113·8°. *For curve* (V), *water* (0·5M) *was also present. For broken lines see text.*

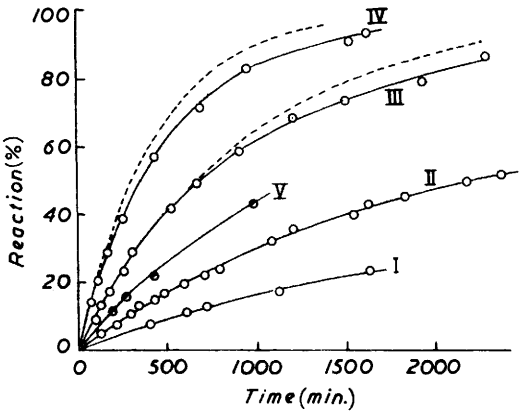
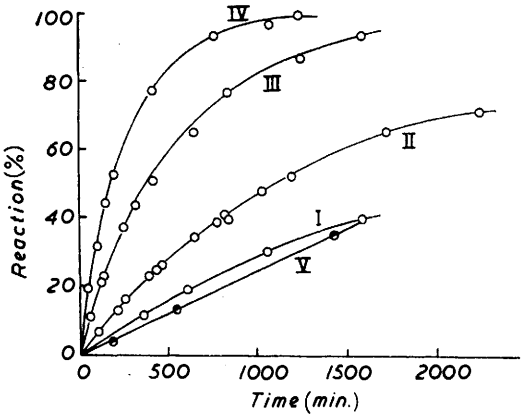


FIG. 2. *Acetolysis of* 0·02M-*cholesteryl iodide in* 0·05M-*solutions of diphenylguanidinium acetate in acetic acid at* (I) 83·8°, (II) 93·8°, (III) 103·8°, *and* (IV) 113·8°. *For curve* (V), *potassium iodide* (0·03M) *was also present.*



V, Fig. 1) to $6{\cdot}3 \times 10^{-4}$ min.$^{-1}$, a value nearly equal to that ($7{\cdot}0 \times 10^{-4}$ min.$^{-1}$) found for 0·05M-diphenylguanidinium acetate solution (curve II, Fig. 2).

The results obtained when anhydrous acetic acid was used as solvent at 93·8° and 113·8°, given in Tables 3 and 4, exhibit features similar to those observed with cholesteryl bromide[3] and epicholesteryl bromide.[4] The reaction proceeds to an equilibrium, and the sigmoid form of the percentage reaction–time plot (curve IV, Fig. 3) indicates that

TABLE 3. *Acetolysis of* 0·02M-*cholesteryl iodide* ($10^5a = 10{\cdot}15$) *in anhydrous acetic acid at* 93·8° ± 0·2°.

Time (min.)	10^5x	$10^2x/a$	10^4k (min.$^{-1}$)	Time (min.)	10^5x	$10^2x/a$	10^4k (min.$^{-1}$)
95	0·15	1·5	1·6	457	7·83	77·0	32·2
187	0·67	6·6	3·6	548	7·90	78·7	27·4
197	1·07	10·6	5·9	596	8·63	84·8	31·7
227	2·88	28·4	14·7	663	8·95	88·1	32·1
270	4·68	46·1	22·9	723	8·78	86·4	27·6
346	6·60	65·0	30·3	868	9·05	89·1	25·6
395	7·25	71·4	31·7	894	9·26	91·0	27·1

the reaction is autocatalysed. By analogy with the behaviour observed for cholesteryl bromide,[3] it may be presumed that the catalysis is due to hydrogen ions resulting from the

[7] Hughes, Ingold, *et al.*, *J.*, 1940, 960 *et seq.*; 1952, 2488.

liberated hydrogen iodide; this view is supported by the fact that the catalysis is more marked with cholesteryl iodide, which is consistent with the greater strength of hydrogen iodide than of hydrogen bromide in acetic acid. Whereas for cholesteryl bromide the

TABLE 4. *Acetolysis of* 0·02M-*cholesteryl iodide* ($10^5a = 10{\cdot}15$) *in anhydrous acetic acid at* 113·8° ± 0·2°.

Time (min.)	10^5x	$10^2x/a$	10^4k (min.$^{-1}$)	Time (min.)	10^5x	$10^2x/a$	10^4k (min.$^{-1}$)
20	0·31	3·0	15·4	35	1·89	18·6	58·7
30	0·82	8·0	28·0	50	3·69	36·3	90·2
30	0·87	8·5	29·8	90	4·85	47·7	69·5
35	1·58	15·6	48·2				

equilibrium corresponding to 85% reaction is attained in 950 min. at 94·8°, for cholesteryl iodide the equilibrium corresponding to 90% reaction is reached after only 870 min. at 93·8° despite the fact that the initial specific rate constant for the iodide is $1{\cdot}8 \times 10^{-4}$ min.$^{-1}$ compared with $2{\cdot}5 \times 10^{-4}$ min.$^{-1}$ for the bromide.[3] The solutions remained colour-

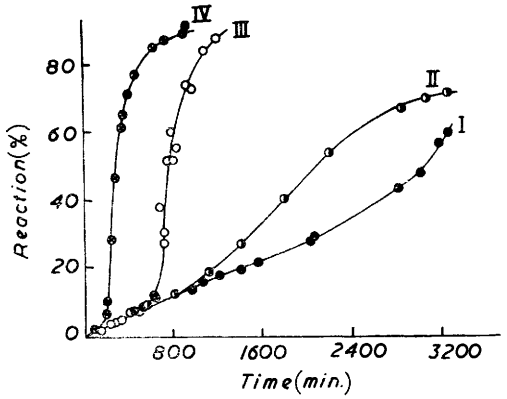


FIG. 3. *Acetolysis of* 0·02M-*cholesteryl iodide at* 93·8° *in* (I) 100% *acetic acid; and in acetic acid containing* (II) 0·1M-, (III) 0·5M-, *and* (IV) 1·0M-*acetic anhydride.*

less for about 200 min.; the subsequent darkening interfered increasingly with the titration procedure and rendered study in the equilibrium region difficult.

Assuming that the acetolysis, in the absence of added acetate, occurs by the mechanism:

$$RI \rightleftharpoons R^+ + I^- \xrightarrow{AcOH} ROAc + HI$$

and that any elimination or other side reaction does not influence the rate, we may write:[3]

$$ak_1t = \ln x/[a(1 - x/x_e)] + c \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where a is the initial molar concentration of cholesteryl iodide, x the extent of reaction at time t, k_1 the rate constant of the catalysed ionisation of the iodide, k_2 the rate constant of the reverse reaction, and x_e the concentration of hydrogen iodide at equilibrium. The observed value of x_e is $0{\cdot}90a$, so that the plot of log $[x/(a - x/0{\cdot}90)]$ against time should be linear. However, two straight lines intersecting at 300 min. were obtained, as in the case of epicholesteryl bromide.[4] Thus it appears the autocatalytic mechanism is fully operative only for the early stage of the reaction; possibly, some cholesta-3,5-diene is formed and by addition of hydrogen iodide reduces the catalytic hydrogen-ion concentration.

The influence of acetic anhydride on the kinetics of acetolysis, previously observed for cholesteryl and epicholesteryl bromide, was found to be less marked in the present study. As shown in Fig. 3, the presence of varying amounts of acetic anhydride leads to the same initial specific rate constant for the acetolysis of 0·02M-cholesteryl iodide at 93·8° as in pure acetic acid. Similarly, the same initial specific rate constant $7{\cdot}0 \times 10^{-4}$ min.$^{-1}$ was

found for the acetolysis of 0·2M-cholesteryl iodide at 113·8° in the presence of 1·0M-acetic anhydride and in pure acetic acid. Clearly, in the early stages of the reaction, acetic anhydride must prevent catalysis by removing hydrogen iodide; but, contrary to the complete inhibition of catalysis, independently of the concentration of acetic anhydride, observed with cholesteryl and epicholesteryl bromide, the extent of inhibition is now dependent on the amount of anhydride present. It was noted that the solutions remained colourless during the period of inhibition, but eventually became highly coloured, so that it was not possible to determine the position of equilibrium in the presence of acetic anhydride.

An explanation of the inhibition of catalysis by acetic anhydride follows from the suggestion [8] that in acetic acid–acetic anhydride solvated protons produce acetylium ions:

$$AcOH_2 + Ac_2O \rightleftharpoons Ac^+ + 2AcOH \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

Acetyl iodide will be formed, but it is known [9] that acetyl chloride and bromide behave as monobasic acids in acetic acid solution and can be titrated with sodium acetate; it is therefore reasonable to assume that conversion of hydrogen iodide into acetyl iodide would not interfere with the titration. The order of decreasing basicity of the anions in acetic anhydride is: $AcO^- > Cl^- > Br^- > I^-$.[9] Thus, the reaction $Ac^+ + X^- \rightleftharpoons AcX$ is less complete for I^- than for Br^-. The position of equilibrium in reaction (2) will therefore lie more to the left for solutions of hydrogen iodide than for solutions of hydrogen bromide; this circumstance, together with the greater strength of hydrogen iodide, may account for the much weaker inhibition of catalysis in the case of cholesteryl iodide. Temperature also influences the inhibitory power of acetic anhydride; at 113·8°, autocatalysis set in practically from the commencement of reaction, whereas at 93·8° it was delayed until after 2000 min.

The energies of activation, calculated from the initial rates at 83·8°, 93·8°, 103·8°, and 113·8° are 23·1 and 22·3 kcal./mole for 0·02M- and 0·05M-diphenylguanidinium acetate solutions respectively. The corresponding entropy changes are −21·7 and −22·5 cal. mole^{-1} deg.$^{-1}$ for the 0·02M- and 0·05M-solutions respectively. The activation energy of 19·2 kcal./mole for the acetolysis in the absence of added base is less reliable since it is computed from measurements at 93·8° and 113·8° only; moreover, development of colour in the very early stages of reaction at 113·8° made titrations difficult, thereby introducing an uncertainty into the value of the initial rate constant.

TABLE 5. *Parameters of acetolyses.*

	Cholesteryl toluene-*p*-sulphonate [2,3]	epi-Cholesteryl toluene-*p*-sulphonate [4]	Cholesteryl bromide [3]	epi-Cholesteryl bromide [4]	Cholesteryl iodide
E (kcal. mole^{-1})	25·0	25·2	26·5	26·8	22·7
$\Delta H^{\ddagger}$ (kcal. mole^{-1})	24·4	24·6	25·8	26·1	22·0
$\Delta S^{\ddagger}$ (cal. mole^{-1} deg.$^{-1}$) ...	−1·0	−3·7	−13·5	−14·5	−22·1

In Table 5 we compare the values of the energy of activation, the heat of activation, and the entropy of activation for the acetolysis of various cholesteryl and epicholesteryl esters. The energy of activation for the iodide is 3—4 kcal. mole^{-1} lower than for the other esters; it must be appreciated that the value for the iodide was obtained under conditions of greater ionic strength, which may contribute to lower the activation energy, but nevertheless the smaller value for the iodide is consistent with the greater ease of heterolysis of the carbon–iodine bond. A survey [10] of unimolecular rates has shown that secondary iodides

[8] Mackenzie and Winter, *Trans. Faraday Soc.*, 1948, **44**, 159.
[9] Usanovitch and Yatsimirsku, *J. Gen. Chem. U.S.S.R.*, 1941, **11**, 954, 959.
[10] Ingold, "Structure and Mechanism in Organic Chemistry," G. Bell and Son, Edinburgh, 1953, p. 306.

ionise 1·5—4·5 times faster than secondary bromides. The observed slower acetolysis of cholesteryl iodide is therefore anomalous, and the greater entropy of activation for the iodide suggests that solvation plays a more significant rôle in the ionisation of the iodide.

The reaction product of the 24 hr. acetolysis of cholesteryl iodide in the presence of added base at 93·8° was cholesteryl acetate (90%) with cholesta-3,5-diene (~10%); 3,5-cyclo-5α-cholestan-6β-yl acetate was not detected. In the absence of added base, only some 20% of cholesteryl acetate was isolated (as cholesterol, after alkaline hydrolysis) although the kinetic measurements indicate 30% conversion; cholesteryl iodide (44%) was recovered, together with unidentified non-crystalline material.

Experimental

For general experimental directions, see *J.*, 1959, 630.

$[\alpha]_D$ are for $CHCl_3$ solutions, and ultraviolet absorption spectra for EtOH solutions measured on a Unicam S.P. 500 spectrophotometer with a corrected scale.

Cholesteryl Iodide.—Cholesteryl toluene-*p*-sulphonate, double m. p. 126°/134°, was converted by methanolysis in the presence of potassium acetate into 6β-methoxy-3,5-cyclocholestane, m. p. 76—77°, $[\alpha]_D$ −46°.[11] This (20 g.) in ether (500 ml.) and acetic acid (200 ml.) was treated with hydriodic acid (" AnalaR "; *d* 1·7, 57% w/w; 200 ml.) overnight at 20°, to give, after the usual isolation procedure, cholesteryl iodide (10 g.), m. p. 107°, $[\alpha]_D$ −14°, after three recrystallisations from anhydrous acetone (lit.,[12] m. p. 107—108°, $[\alpha]_D$ −12°. To minimise decomposition, the iodide was kept in a dark bottle in a desiccator; however, for reproducible rate measurements, periodical recrystallisation from acetone was necessary.

Acetic Acid.—Initially, anhydrous acetic acid for kinetic work was obtained by the procedure of Eichelberger and LaMer,[13] with triacetyl borate prepared by the method of Pictet and Geleznoff.[14] Later, it was found by titration with the Karl Fischer reagent that fresh samples of " AnalaR " acetic acid had a negligible water content and yielded the same reaction rates as did acetic acid dried with triacetyl borate.

Diphenylguanidine.—The commercial reagent grade was recrystallised three times from ethanol and dried in a vacuum-desiccator, then having m. p. 147—148°.

Titrations in Anhydrous Acetic Acid.—An approximately 0·05M-solution of perchloric acid in acetic acid, employed as a standard acid, was prepared from 60% w/w perchloric acid of known titre with addition of the calculated amount of acetic anhydride to remove the water. This perchloric acid solution was standardised against a solution of potassium hydrogen phthalate in acetic acid, with a saturated solution of Crystal Violet as indicator; the end-point involved a sharp colour change from blue to green. Solutions of hydriodic acid in acetic acid were titrated by adding a known volume of standard diphenylguanidinium acetate solution and determining the excess of base by titration against standard perchloric acid. The validity of this procedure was confirmed by using a standard solution of hydrogen iodide in acetic acid obtained from freshly prepared constant-boiling hydriodic acid, the water of which had been removed by treatment with the calculated amount of acetic anhydride. The perchloric acid solution was standardised against the diphenylguanidinium acetate solution before each batch of titrations.

Rate Measurements.—The temperature of the thermostat-bath was maintained within a few degrees of the required value by an immersion heater, temperature control being by an auxiliary heater of lower wattage coupled with a mercury–xylene thermoregulator and a " Sunvic " (E.A.3) electronic relay. The thermostat was set at 83·8°, 93·8°, 103·8°, and 113·8° severally, by means of a certified N.P.L. thermometer; temperature control was within ±0·2°.

Preliminary rate measurements were carried out by the sealed ampoule procedure described by Davies, Meecham, and Shoppee.[3] Approximately 50 mg. of the iodide, weighed to the nearest 0·01 mg., were introduced into an ampoule together with 5 ml. of solvent. After the ampoule had been sealed and immersed in the bath, the solution rapidly darkened and location of the end-point in the subsequent titration of the liberated acid became very difficult. The following degassing procedure was adopted.

[11] Stoll, *Z. physiol. Chem.*, 1932, **207**, 147.
[12] Broome, Brown, and Summers, *J.*, 1957, 2071.
[13] Eichelberger and LaMer, *J. Amer. Chem. Soc.*, 1933, **55**, 3633.
[14] Pictet and Geleznoff, *Ber.*, 1903, **36**, 2219.

Ampoules, made from tubing of ~1·5 cm. diameter, were provided with a standard B-19 cone for attachment to a vacuum-line. Since the solubility of cholesteryl iodide in acetic acid is too low at room temperature to permit a stock standard solution of suitable strength to be used, solutions were made up separately in each ampoule. After addition of a weighed amount of the iodide to the ampoule, the solid was washed down the tube by addition of 5 ml. of solvent from a pipette. The weight of iodide taken was such that the solution was as nearly as possible 0·02M. For each batch of solutions made up at one time, a 5 ml. portion of the solvent was weighed, to allow the strength of the solutions to be expressed on a molality basis and to correct for any volume changes of the solvent due to slight variations in room temperature. The contents of each ampoule were frozen in acetone–solid carbon dioxide, and the tube then constricted at about 10 cm. from the cone to facilitate subsequent sealing.

After attachment of the ampoule to the vacuum-line and again freezing of its contents, the tube was evacuated to ~10^{-2} mm., the extent of evacuation being ascertained by the strength of the discharge obtained with a " Tesvac " coil. The ampoule was then isolated from the vacuum-line by a tap and the solid solution allowed to melt. This solution was again frozen and the evacuation repeated. The sequence of melting, freezing, and evacuation was carried out a third time to ensure complete degassing of the solution. With the solution frozen, the ampoule was sealed at the constriction. Controls showed that loss of solvent was less than 0·02 g. in the above procedure.

Dissolution of the iodide was complete after the ampoule had been immersed for approximately 2 min. in the bath. After an appropriate interval the reaction was stopped by immersing the ampoule in ice-water. The contents of the tube were washed into a flask with anhydrous acetic acid and analysed. In runs carried out in the absence of added base, the ampoules were broken under a measured volume of standard base solution to prevent loss of hydrogen iodide and were then washed out with anhydrous acetic acid. It was necessary to perform the titrations immediately the ampoule was opened, since if the solution was exposed to air for any length of time the end-point was affected, presumably by oxidation of the diphenylguanidinium iodide. The decomposition was catalysed by direct sunlight and appropriate precautions were taken.

A 0·02M-solution of hydrogen iodide in anhydrous acetic acid, degassed by the above procedure, underwent negligible decomposition after 36 hr. at 93·8°.

Determination of Products.—(a) *In presence of added base.* A 0·02M-solution of cholesteryl iodide (50 ml.) in a 0·05M-solution of diphenylguanidinium acetate in acetic acid in a large ampoule was degassed, sealed, kept at 113·8° for 24 hr., and then was poured into water. The product was extracted with ether and recovered in the usual way as a solid (436 mg.), which melted over a wide range; it was chromatographed on a column of aluminium oxide (Spence, type H; 15 g.) prepared in pentane. Elution with pentane (2 × 50 ml.) gave cholesta-3,5-diene (33 mg.), m. p. and mixed m. p. 80°, $\lambda_{max.}$ 235 mμ. Further elution with pentane (26 × 50 ml.) gave cholesteryl acetate (325 mg.), m. p. and mixed m. p. 115°; ether–benzene (1 : 9) (11 × 50 ml.) gave cholesterol (57 mg.), m. p. and mixed m. p. 148°.

(b) *In absence of added base.* A 0·02M-solution (100 ml.) of cholesteryl iodide in a 1·0M-solution of acetic anhydride in acetic acid in a large ampoule was degassed, sealed, and kept at 93·8° for 36 hr., after which the solution was faintly coloured. Chromatography of the product, isolated as above, failed to effect separation. The procedure was repeated and the product refluxed with 5% methanolic potassium hydroxide for 0·5 hr.; the hydrolysed product, isolated in the usual manner, was a dark brown oil (915 mg.) and was chromatographed on aluminium oxide (30 g.) in pentane. Elution with pentane (2 × 100 ml.) afforded a colourless oil (221 mg.), $\lambda_{max.}$ 213 mμ, giving a positive halogen test and probably consisting mainly of cholesteryl iodide; further elution with pentane (3 × 100 ml.) gave cholesteryl iodide (186 mg.), m. p. and mixed m. p. 107°. Use of benzene–pentane (1 : 9; 5 × 100 ml.) gave impure cholesteryl iodide (256 mg.), whilst benzene (5 × 100 ml.) and ether–benzene (1 : 19; 4 × 100 ml.) gave brown oils showing a positive halogen test (total, 97 mg.). Final elution with ether gave cholesterol (84 mg.), m. p. 148—149°.

(c) *Stability of epicholesteryl acetate in anhydrous acetic acid.* A solution of epicholesteryl acetate (256 mg.) and diphenylguanidine (260 mg.) in glacial acetic acid (25 ml.) was kept at 93·8° for 36 hr., then neutralised with sodium carbonate and extracted several times with benzene. The benzene extract was dried (Na_2SO_4), and the residue (249 mg.) after evaporation was boiled with lithium aluminium hydride for 1 hr. in ether. The product (224 mg.) was

identified, after chromatography on alumina, as epicholesterol, m. p. and mixed m. p. 144—145°, depressed by cholesterol (acetate, m. p. and mixed m. p. 82—83°). No cholesta-3,5-diene was isolated on prior elution with pentane.

We thank Glaxo Laboratories Limited, London, for a gift of cholesterol; one of us (W. C.) gratefully acknowledges the financial support of the Swansea Local Education Authority.

UNIVERSITY COLLEGE, SWANSEA; THE UNIVERSITY OF WALES.
DEPARTMENT OF ORGANIC CHEMISTRY, THE UNIVERSITY OF SYDNEY,
N.S.W., AUSTRALIA. [*Received, December 2nd,* 1959.]