Steroids and Walden Inversion. Part XX. A Kinetic Study of the Acetolysis of Cholesteryl Bromide.*

By R. H. DAVIES, SYLVIA MEECHAM, and C. W. SHOPPEE.

[Reprint Order No. 5636.]

Acetolysis of cholesteryl bromide in the presence of added acetate has been shown to proceed by a unimolecular heterolysis, thereby confirming the view (Shoppee, *J.*, 1946, 1147) that 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, but it appears that the rate-determining stage is the catalysed ionisation of the bromide. In the presence of acetic anhydride, the reaction gives a first-order rate constant which is insensitive to the concentration of anhydride over a wide range. An explanation of this result is offered. $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values for the reaction are compared with the corresponding values for the acetolysis of cholesteryl toluene-*p*-sulphonate; it is concluded with Simonetta and Winstein (*J. Amer. Chem. Soc.*, 1954, **76**, 18) that stabilisation of the carbonium ion resulting from the heterolysis by the π-electrons of the 5 : 6-double bond is of the order of a few kcal. mole^{-1} only.

EARLIER (Shoppee, *J.*, 1946, 1147) it was shown that acetolysis of cholesteryl chloride occurred with complete retention of configuration at $C_{(3)}$. This behaviour, which was shown by configurational studies to be typical of replacement reactions with weakly nucleophilic reagents at $C_{(3)}$ in Δ^5-steroids, is in marked contrast to the corresponding reactions at $C_{(3)}$ in saturated steroids, which proceed with inversion of configuration (*idem, ibid.*, p. 1138). It was suggested that for nucleophilic substitution reactions at $C_{(3)}$ in Δ^5-steroids the π-electrons of the 5 : 6-double bond not only facilitate ionisation of the atom or group attached to $C_{(3)}$ leading to a unimolecular heterolysis, but also preserve configuration at $C_{(3)}$ (sp^3-hybridisation with a vacant orbital) in the resulting carbonium ion, by interaction (partly electrovalent, partly covalent) with the positive charge. This proposed mechanism has been examined in greater detail more recently by Shoppee and Summers (*J.*, 1952, **3361**) in relation to the **3** : 5-*cyclo*steroid rearrangement, whilst Simonetta and Winstein (*J. Amer. Chem. Soc.*, 1954, **76**, 18) have applied a molecular-orbital treatment to estimate the stabilisation due to the π-electron delocalisation.

* Part XIX, *J.*, 1954, 4224, in line 13 of which "kinetic" and "thermodynamic" should be interchanged.

Winstein and Adams (*ibid.*, 1948, **70**, 838) reported that acetolysis of cholesteryl toluene-*p*-sulphonate in anhydrous acetic acid either alone or in the presence of added acetate, gave first-order rate constants; Wallis, Hafez, and Halsey (*Science*, 1949, **110**, 474) also found a first-order rate constant for the hydrolysis of cholesteryl toluene-*p*-sulphonate in aqueous acetone in the presence of acetate ion and showed that the rate of hydrolysis increased with increasing ease of separation of the 3β-substituent as the anion: p-$C_6H_4Me{\cdot}SO_3^- < Ph{\cdot}SO_3^- <$ p-$NO_2{\cdot}C_6H_4{\cdot}SO_3^-$, thereby providing experimental confirmation of a mechanism of S_N1 type. It was of interest to ascertain whether the cholesteryl halides would behave similarly on acetolysis and to obtain additional data which might contribute to a more detailed analysis of the reaction mechanism.

Preliminary observations showed that acetolysis of the more readily available cholesteryl chloride was inconveniently slow for a detailed kinetic study. Thus even at 95° the reaction in the absence of added acetate was only about one-third complete after 72 hours. Moreover difficulty was experienced in following the course of the reaction by titrating the liberated hydrogen chloride. Contrary to the work of Steigman and Hammett (*J. Amer. Chem. Soc.*, 1937, **59**, 2536) it was found that the titration of approximately 0·02M-solutions of hydrogen chloride in acetic acid against sodium acetate as a base could not be effected with any degree of accuracy when crystal-violet was used as indicator. Further, no sharp end-point could be observed on back-titration although a number of other indicators covering a wide range of pH values was tried. This difficulty may be attributed to the low strength of hydrogen chloride in acetic acid solution. Smith and Elliot (*ibid.*, 1953, **75**, 3566) have calculated the dissociation constants of certain acids in acetic acid solution from the conductance results obtained by Kolthoff and Willman (*ibid.*, 1934, **56**, 1007). The dissociation constants of perchloric, hydrobromic, sulphuric, and hydrochloric acid were computed to be 9×10^{-7}, $1{\cdot}9 \times 10^{-7}$, $7{\cdot}4 \times 10^{-9}$, and $5{\cdot}1 \times 10^{-10}$ respectively. These values give the approximate relative strengths of the four acids as $HClO_4 : HBr : H_2SO_4 : HCl =$ 42 : 19 : 4 : 1. Shkodin and Izmailov (*J. Gen. Chem., U.S.S.R.*, 1950, **20**, 39) have reported slightly higher values for the dissociation constants of these acids but the relative strengths are the same as those already quoted. It is evident that hydrogen chloride is a very weak acid in acetic acid solution and that chloride solutions are extensively solvolysed.

Acetolysis of cholesteryl bromide was found to be more convenient for study since at 95° the reaction was about half complete after seven hours and, as a consequence of the

TABLE 1. *Acetolysis of approx.* 0·02M-*solutions of cholesteryl bromide in the presence of* 0·05M-*diphenylguanidinium acetate, at* 94·8° ± 0·15°.

Time (min.)	10^2M	Reaction (%)	10^3k_1 (min.$^{-1}$)	Time (min.)	10^2M	Reaction (%)	10^3k_1 (min.$^{-1}$)
90	2·018	13·7	1·63	420	2·089	41·4	1·27
120	2·106	16·5	1·50	705	2·039	56·9	1·20
150	2·144	21·7	1·63	900	2·037	62·6	1·09
200	2·008	25·5	1·47	1080	2·132	66·2	1·02
240	1·888	29·0	1·43	1200	2·108	70·3	1·01
247	2·082	28·4	1·35	1440	2·133	73·7	0·93
300	2·045	33·5	1·36	1669	2·135	76·9	0·88
360	2·156	40·2	1·43	1800	2·126	78·3	0·85

TABLE 2. *Acetolysis of approx.* 0·02M-*solutions of cholesteryl bromide in the presence of* 0·02M-*diphenylguanidinium acetate, at* 94·8° ± 0·15°.

Time (min.)	10^2M	Reaction (%)	10^3k_1 (min.$^{-1}$)	Time (min.)	10^2M	Reaction (%)	10^3k_1 (min.$^{-1}$)
90	2·100	9·6	1·12	300	2·156	23·1	0·88
129	2·129	13·0	1·08	900	2·047	44·0	0·65
180	2·048	16·0	1·21	1440	2·079	55·3	0·56

greater strength of hydrogen bromide in acetic acid, no difficulty was experienced in following the reaction by titration of the liberated acid. A selection of results obtained for the acetolysis of approximately 0·02M-cholesteryl bromide in 0·05M- and 0·02M-diphenylguanidinium acetate solution in anhydrous acetic acid at 94·8° ± 0·15° is presented in Tables 1 and 2. For small intervals, the experimental limitation of ±0·01 ml. in titration of the liberated hydrogen bromide introduces an uncertainty of about ±5% in the individual

values of k, but this becomes progressively less as the duration of the reaction increases. From Tables 1 and 2, it will be seen that within the limits of experimental error the reaction, in its early stages, does not deviate significantly from the usual first-order kinetic relation $dx/dt = k_1x(a - x)$ but, as the reaction proceeds, the value of the specific rate constant becomes progressively less. From these results, the initial specific rate constants (obtained by extrapolation to zero reaction) in the presence of 0·05M- and 0·02M-diphenylguanidinium acetate are $1·75 \times 10^{-3}$ and $1·25 \times 10^{-3}$ min.$^{-1}$ respectively. When the acetolysis was effected in the presence of sodium acetate instead of diphenylguanidinium acetate, the initial first-order specific rate constants for 0·05M- and 0·02M-solutions of sodium acetate were $0·70 \times 10^{-3}$ and $0·61 \times 10^{-3}$ min.$^{-1}$ respectively. Thus, the rate is different in the presence of the same concentration of different acetates (cf. Steigman and Hammett, *loc. cit.*). As will be seen later, the reaction in the pure solvent is complicated by autocatalysis, but the initial specific rate constant is $0·25 \times 10^{-3}$ min.$^{-1}$; increasing the concentration of added acetate ions thus accelerates the reaction and, when the influences of diphenylguanidinium and sodium acetates are compared, the effect is seen to depend on the nature of the added electrolyte. We are of the opinion that these results are explicable on the basis that the acetolysis involves the following unimolecular heterolysis :

$$RBr \underset{(2)}{\overset{(1)}{\rightleftharpoons}} R^+ + Br' \xrightarrow[(3)]{Ac'} RAc + Br'$$

Hughes, Ingold, *et al.* (*J.*, 1940, 960 *et seq.*; 1952, 2488) have shown that the reversible nature of the rate-determining ionisation step (1) leads to a progressive retardation of the reaction irrespective of whether the overall reaction is reversible or otherwise. As the reaction proceeds, the concentration of bromide ion resulting from stage (3) increases, thereby causing stage (2) to become more significant and to diminish the rate. In such circumstances, it is necessary to employ the modified kinetic expression $dx/dt = k(a - x)/(1 + \alpha x)$, where α is a factor which determines the ability of bromide ion to compete with acetate ion for the carbonium ion R^+ and the factor $(1 + \alpha x)$ determines the extent of departure from the specific rate $(dx/dt)/(a - x)$. Taking α to be approximately 50 is adequate to compensate for the downward drift in the values of k_1 given in Table 1.

We attribute the effect of added acetate ion on the rate of the reaction to the variation in the ionic strength of the solvent. The low dielectric constant of acetic acid (6 at 25° and less at 95°) favours extensive ionic association in this solvent (Fuoss and Kraus, *J. Amer. Chem. Soc.*, 1933, **55**, 1019). Since the extent of ion association increases markedly with decreasing ionic diameter, this would account for the difference in rate observed in the presence of diphenylguanidinium acetate and sodium acetate. The latter electrolyte is less dissociated and consequently gives rise to a smaller ionic-strength effect. Similar specific-salt effects have been observed by Steigman and Hammett (*loc. cit.*) for the acetolysis of 1-phenylethyl halides. In an attempt to compensate for the lower ionic strengths of the solutions in Table 2, some of the measurements were repeated in the additional presence of 0·03M-diphenylguanidinium perchlorate. The specific rate constant under these conditions increased to $2·52 \times 10^{-3}$ min.$^{-1}$, so that the ionic-strength effect resulting from the addition of 0·03M-diphenylguanidinium perchlorate is greater than that from 0·03M-diphenylguanidinium acetate. This specific-salt effect is again a consequence of the disparity between the dissociation constants of the two electrolytes.

The reaction products * in the presence of added acetate were established by chromatographic analysis as consisting of cholesteryl acetate and unchanged cholesteryl bromide in amounts which agreed with the rate observations.

The reaction in acetic acid alone presented some unexpected features. From Table 3 it will be observed that the rate of acetolysis increases with time until the reaction is approximately two-thirds complete and then diminishes until a state of equilibrium is

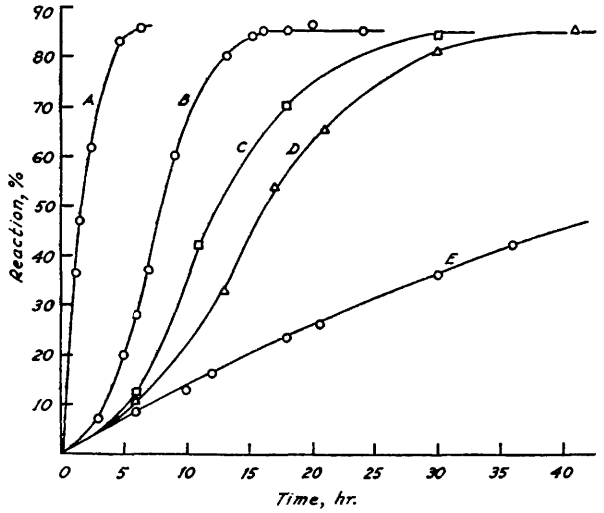
* The acetolysis could lead to the production in reaction (2) of 3 : 5-*cyclo*cholestan-6β-yl bromide when carried out in the presence of added acetate ion or of acetic anhydride, *i.e.*, in the absence of hydrogen bromide, to which as a strong acid 3 : 5-*cyclo*steroids are labile (cf. Shoppee and Summers, *J.*, 1952, 3361, especially 3368); such a process would be kinetically equivalent to the production of cholesteryl bromide in the reassociation process (2), but no such compound could be isolated.

reached corresponding to 85% conversion. These results are represented graphically by curve *B*. Curves *C* and *D* represent the corresponding results obtained for approximately 0·01M- and 0·005M-solutions of bromide respectively. The three curves show that the reaction proceeds to the same equilibrium and their sigmoid form, especially for the more

TABLE 3. *Acetolysis of approx.* 0·02M-*solutions of cholesteryl bromide at* 94·8° ± 0·15°.

Time (min.)	(10^2M)	Reaction (%)	10^3k_1 (min.$^{-1}$)	Time (min.)	(10^2M)	Reaction (%)	10^3k (min.$^{-1}$)
180	1·878	7	0·41	918	2·101	84	2·00
300	1·953	20	0·74	946	2·037	85	2·01
360	1·904	28	0·92	1080	1·900	85	1·76
410	2·085	37	1·13	1200	2·054	86	1·64
540	2·040	60	1·69	1440	2·053	85	1·32
787	2·055	80	2·03	2468	1·989	85	0·76

concentrated solution, indicates that the reaction is autocatalysed. The discrepancy between the initial specific rate constant of $0{\cdot}25 \times 10^{-3}$ min.$^{-1}$ observed under these conditions and the corresponding values obtained in the presence of added acetate is of the order of magnitude expected from an ionic-strength effect. Confirmation of the fact that the hydrogen bromide liberated during the reaction was responsible for the catalysis was



A, *Acetolysis of* 0·02M-*cholesteryl bromide in presence of* 0·017M-*hydrobromic acid.*
B, *Acetolysis of* 0·02M-*cholesteryl bromide.*
C, *Acetolysis of* 0·01M-*cholesteryl bromide.*
D, *Acetolysis of* 0·005M-*cholesteryl bromide.*
E, *Acetolysis of* 0·02M-*cholesteryl bromide in presence of* 0·1M-*acetic anhydride solution.*

obtained from a series of determinations on 0·02M-solutions of cholesteryl bromide which were initially 0·017M with respect to hydrogen bromide. This initial amount of acid resulted in attainment of equilibrium in about 4 hours as compared with 17 hours in its absence (curve *A*). A similar result was obtained with perchloric acid. On the other hand when the reaction was effected in the presence of 0·01M-tetramethylammonium bromide, only a slight increase in rate was detected. It appears therefore that hydrogen ions are responsible for the catalysis and that these are effectively removed by the addition of acetate ions. The increase by a few per cent. observed in the conversion of the bromide resulting from the presence of 0·5M-water is probably due to the increased ionising tendency under these conditions.

Contrary to the above observations on cholesteryl bromide, Winstein and Adams (*loc. cit.*) reported a good first-order rate constant for the acetolysis of cholesteryl toluene-*p*-sulphonate. Moreover, although the reaction was studied until it was about 70% complete, there was no indication that the reaction reached an equilibrium. Re-examination of the kinetics of this reaction at 50° gave a first-order constant of $7{\cdot}5 \times 10^{-3}$ min.$^{-1}$ which agrees with the value of $7{\cdot}9 \times 10^{-3}$ min.$^{-1}$ obtained by Winstein and Adams. The specific rate constant was found to show a slight downward drift with time but, although the reaction was studied until it was 97% complete, there was no evidence either of catalysis or that an equilibrium had been attained.

When the acetolysis of 0·02M-solutions of cholesteryl bromide was carried out in the presence of 0·017M-toluene-*p*-sulphonic acid, no significant change in the rate could be detected. Although toluene-*p*-sulphonic acid is weaker than either perchloric or hydrobromic acid in acetic acid solution (Shkodin and Ismailov, *loc. cit.*), it is difficult to understand the marked difference in behaviour of the bromide and the toluene-*p*-sulphonate. Shoppee and Williams (following paper) have also observed this anomalous behaviour in the acetolysis of *epi*cholesteryl bromide.

A further complication is that, under the conditions employed for acetolysis, the liberated hydrogen bromide can effect decomposition of the steroid molecule, as is shown by the solutions' gradually developing a brown colour.* Chromatographic analyses of the products obtained under these conditions were inconclusive on account of the difficulty experienced in crystallising the oils eluted. The ultra-violet absorption band observed at 213 mμ for ethanolic solutions of these oils probably corresponds to the presence of cholesteryl bromide (Bladon, Henbest, and Wood, *J.*, 1952, 2737), whilst the broad band in the 240-mμ region may be attributed to the presence of cholesta-3 : 5-diene (see Table 25; Dorfman, *Chem. Reviews*, 1953, **53**, 108) formed by elimination. Cholesteryl acetate was found to react with hydrogen bromide in acetic acid solution to give some cholesteryl bromide, thus accounting for the observation made from the kinetic measurements that an equilibrium was established.

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

$$RBr \rightleftharpoons R^+ + Br' \overset{AcOH}{\rightleftharpoons} RAc + HBr$$

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

$$dx/dt = k_1(a - x)x - k_2x^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where a is the initial concentration of cholesteryl bromide, x the extent of reaction at time t, $k_1(a - x)x$ is the rate of the catalysed ionisation of the bromide, and k_2x^2 the rate of the back reaction. Writing x_e for the concentration of hydrogen bromide at equilibrium, we get :

$$k_1(a - x_e)x_e = k_2x_e^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

When equation (2) is used to relate the coefficients k_1 and k_2, the integrated form of equation (1) becomes :

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

The experimentally observed value of x_e is $0{\cdot}85a$. The plot of $\log [x/(a - x/0{\cdot}85)]$ against time was found to be linear. Thus the mechanism envisaged for the reaction appears to be satisfactory and the rate-determining step is the ionisation of the bromide, catalysed by hydrogen ions, the overall reaction being reversible.

During the preliminary investigation of the reaction, the above autocatalysis was found to be absent when a particular specimen of acetic acid was employed as solvent. The

TABLE 4. *Acetolysis of cholesteryl bromide in the presence of acetic anhydride. Temp.* 94° ± 0·15°.

Concn. of bromide (10^3M)	1·892	1·921	1·873	1·823	1·816	2·068	1·978
Concn. of anhydride (M)	0·05	0·10	0·25	0·5	1·0	60%	100%
Time (min.)	1230	1230	1835	1020	1230	1528	1650
Reaction (%)	26·7	25·7	36·7	22·7	27·7	35·8	0
10^3k_1 (min.$^{-1}$)	0·252	0·242	0·250	0·252	0·263	0·290	—

difference in behaviour was subsequently traced to the presence of acetic anhydride as an impurity in this solvent. A systematic study of the effect of acetic anhydride on the rate of acetolysis of cholesteryl bromide revealed, as shown in Table 4, that the rate was independent of the concentration of acetic anhydride up to 1M. Even in a solvent containing

* Work in progress has shown that traces of molecular oxygen in the solution influence the development of colour.

60% of acetic anhydride, the change in rate was not more than expected from such a change in the dielectric constant and other properties of the solvent.

Each run made in a solvent containing acetic anhydride gave an excellent first-order rate constant. Since the initial specific rate constant for acetolysis in the pure solvent ($0{\cdot}25 \times 10^{-3}$ min.$^{-1}$) is the same as the rate constant observed in solvent containing acetic anhydride (see also the Figure), it follows that the latter must effectively remove the hydrogen bromide which is responsible for the catalysis. A plausible explanation for the removal of the catalytic species by acetic anhydride emerges from Mackenzie and Winter's proposal (*Trans. Faraday Soc.*, 1948, **44**, 159) that in acetic acid–acetic anhydride solutions of mineral acids the strongly electrophilic solvated proton, $AcOH_2^+$, reacts with acetic anhydride according to the equation, $AcOH_2^+ + Ac_2O \rightleftharpoons Ac^+ + (AcOH)_2$. The equilibrium would not interfere with the titration procedure since the resulting acetyl bromide is known to behave as a monobasic acid in acetic acid solution and can be titrated against sodium acetate (Usanovitch and Yatsimirsku, *J. Gen. Chem. U.S.S.R.*, 1941, **11**, 954, 959).

Measurements made on the acetolysis of cholesteryl bromide in the presence of 0·1M-acetic anhydride at 84·8° gave a specific rate constant of $9{\cdot}1 \times 10^{-5}$ min.$^{-1}$. Combining this value with the smoothed value of $0{\cdot}25 \times 10^{-3}$ min.$^{-1}$ observed at 94·8° gives 26·5 kcal. mole^{-1} for the energy of activation as calculated according to the Arrhenius equation. It is reasonable to assume that this also represents the energy of activation for the uncatalysed reaction. The heat of activation ($\Delta H^{\ddagger}$) is 25·8 kcal. mole^{-1} and is 1·4 kcal. mole^{-1} greater than that for cholesteryl toluene-*p*-sulphonate; $\Delta H^{\ddagger}$ is possibly in error by 1 kcal. mole^{-1} because it is based on measurements at two temperatures only, but it is evidently greater than the value (24·4) observed for cholesteryl toluene-*p*-sulphonate. The corresponding value for cholestan-3β-yl bromide is not available (see, however, Simonetta and Winstein, *loc. cit.*, p. 20, footnote 23) so that it is not possible at present to ascertain the extent to which the π-electrons of the 5 : 6-double bond have influenced the activation energy. However, from the value of 27·0 kcal. mole^{-1} for *cyclo*hexyl toluene-*p*-sulphonate (Winstein and Adams, *loc. cit.*) it seems permissible to conclude with Simonetta and Winstein that stabilisation of the carbonium ion by the π-electrons is of the order of a few kcal. mole^{-1} only. The entropy of activation ($\Delta S^{\ddagger}$) for cholesteryl bromide is −13·5 kcal. mole^{-1} deg.$^{-1}$, compared with only −1·0 cal. mole^{-1} deg.$^{-1}$ for the toluene-*p*-sulphonate. Since the rate-determining step is one of ionisation, it follows that there should be a parallelism between the entropy of activation and the entropy of solvation of the ions. Evans and Hamann (*Trans. Faraday Soc.*, 1951, **47**, 40) are of the opinion that it is the change in entropies of solvation which largely determines the change in $\Delta S^{\ddagger}$ values. The larger toluene-*p*-sulphonate ion with its more dispersed charge will orientate the solvent molecules to a smaller extent than the bromide ion. This is consistent with the more negative $\Delta S^{\ddagger}$ value observed for the acetolysis of cholesteryl bromide.

Experimental

For general experimental directions, see *J.*, 1954, 4224. $[\alpha]_D$ are in $CHCl_3$, and ultra-violet absorption spectra were determined in EtOH on a Unicam Sp. 500 spectrophotometer with corrected scale.

Anhydrous Acetic Acid.—Preliminary kinetic measurements showed that the rates were uninfluenced by traces of organic impurities present in " AnalaR " acetic acid and the initial procedure involving distillation over chromium trioxide was therefore omitted. The water content of " AnalaR " acetic acid was determined by direct titration with the Karl Fischer reagent; the titration was satisfactory when carried out quickly and gave a result which agreed with the water content calculated from the lowering of the f. p. of the acid on the assumption that water was the only impurity. The water was removed by refluxing the acid for 36—48 hr. with the calculated amount of acetic anhydride in the presence of a little diphenylguanidinium acetate as a catalyst. Fractional distillation of the products yielded a main fraction boiling at 118·0—118·2°. In subsequent operations, precautions were taken to minimise exposure of the solvent to atmospheric moisture.

Cholesteryl Bromide.—This was prepared by the method of Jones *et al.* (*J.*, 1948, 1787). The

thionyl bromide employed was prepared and purified as described in *Inorg. Synth.*, Vol. I, p. 113. Several recrystallisations from acetone gave cholesteryl bromide, m. p. 98°, $[\alpha]_D^{18}$ −21°.

Cholesteryl Toluene-p-sulphonate.—This (m. p. 132°) was prepared as described by Wallis, Fernholz, and Gephart (*J. Amer. Chem. Soc.*, 1937, **59**, 137).

Diphenylguanidine.—Material of reagent grade was recrystallised four times from ethanol; after drying at 120°, it had m. p. 148°.

Acetic Anhydride.—" AnalaR " material was refluxed over marble for 3 hr. and distilled (b. p. 138°).

Titrations in Anhydrous Acetic Acid.—An approximately 0·05M-solution of perchloric acid in acetic acid, employed as a standard acid, was prepared from 70% aqueous acid of known titer, the calculated amount of acetic anhydride being added to remove the water present. The perchloric acid solution was standardised against a solution of potassium hydrogen phthalate in acetic acid. Crystal-violet was found to be a satisfactory indicator. A semimicroburette, reading to 0·01 ml., was employed and titrations were conducted at a uniform room temperature in order to minimise errors in concentration arising from expansion of the solvent.

Solutions of hydrobromic 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 end point with crystal-violet as indicator was sharp and reproducible to 0·01 ml. The titration was confirmed by a differential potentiometric method (MacInnes and Dole, *J. Amer. Chem. Soc.*, 1929, **51**, 119), using platinum electrodes in a saturated solution of quinhydrone in acetic acid. On account of the high electrical resistance of such solutions, an electronic pH meter was employed for recording the e.m.f.

In the corresponding titration of hydrochloric acid solutions in acetic acid, the end point was indefinite, although a variety of indicators covering a wide range of pH values was tried.

Rate Measurements.—The velocity of acetolysis was determined in a thermostat, electronically regulated at 94·8° ± 0·15°. A technical product, " Ucepal " (an ethylene glycol derivative), proved to be a convenient thermostatic liquid at this temperature. The sealed ampoule procedure was adopted in order to minimise losses of solvent and hydrobromic acid from the solutions. The low solubility of cholesteryl bromide in acetic acid at room temperature did not permit the preparation of a stock solution and it was necessary to introduce into each tube, separately, a weighed amount of bromide and a measured volume of solvent to give the desired molarity. The solutions, which occupied a volume of between 5 and 10 ml., were made up by weight in each tube. About 50 mg. of bromide, weighed to the nearest 0·01 mg., were used for each determination. Preliminary measurements showed that the reaction proceeded at a negligible rate at room temperature.

Dissolution of the bromide occurred within 30 sec. when the ampoules were immersed in the thermostat and thoroughly shaken. The solutions attained the temperature of the thermostat within 2 min. The reaction was stopped by immersing the ampoule in ice-cold water. When the solutions contained free hydrobromic acid, loss of acid was avoided by opening the ampoule under a solution containing a known volume of standard diphenylguanidinium acetate, and the excess of base was then determined by titration against perchloric acid.

Acetolysis in the pure solvent involved a darkening of the solution towards the end of the reaction. The development of colour was less marked when the solvent contained added base. In the presence of added acetic anhydride the solutions developed a pink colour within about 15 hr. but after about 48 hr. the solutions had darkened to such an extent as to make their titration more difficult.

Determination of the Reaction Products.—A 0·02M-solution (50 ml.) of cholesteryl bromide in a 0·05M-solution of diphenylguanidinium acetate in acetic acid was sealed in a small flask and immersed for 12 hr. in the thermostat at 95°. The mixture was poured into water and extracted twice with ether. After washing successively with water, dilute acid, water, sodium carbonate, and finally with water until neutral, evaporation of the ether yielded an oil which solidified. The solid was chromatographed on aluminium oxide in pentane, and pentane eluates were found to yield cholesteryl bromide, m. p. 95—96°, whilst elution with benzene–pentane mixtures gave cholesteryl acetate, m. p. 116°. The yield of cholesteryl acetate (56%) agreed quantitatively with the conversion of bromide observed by the rate measurements.

The determination was repeated in the absence of a base. The product was extracted with ether and hydrolysed with 5% methanolic potassium hydroxide for 1·5 hr., and the resulting solution poured into water. Ether-extraction gave an oil, about 20% of which was insoluble in methanol and presumably non-steroidal. The methanol-soluble portion was chromatographed on aluminium oxide in pentane. Elution with pentane gave an oil, $[\alpha]_D$ +51° (*c*, 1·8), $\lambda_{max.}$

213 and 245 mμ, showing a positive test for halogen. Benzene–pentane and benzene eluates yielded traces of oil which could not be crystallised.

Action of Hydrogen Bromide on Cholesteryl Acetate.—An ampoule containing 484 mg. of cholesteryl acetate in 50 ml. of a 0·02M-solution of hydrogen bromide in acetic acid, was kept in a thermostat at 90° for 12 hr. The brown solution was poured into water. Ether-extraction gave a brown oil (452 mg.), which failed to crystallise on nucleation with starting material. The oil was chromatographed on aluminium oxide in pentane. The pentane eluates yielded an oil (356 mg.) which gave a positive Beilstein test and a brown colour in the Rosenheim test. The benzene–pentane and benzene eluates yielded 47 mg. and 17 mg. respectively of brown oil.

Action of Methanolic Potassium Hydroxide on Cholesteryl Bromide.—Cholesteryl bromide (179 mg.) in 100 ml. of 5% solution of potassium hydroxide in methanol was refluxed for 1·5 hr. The product was poured into water and worked up in the usual manner. Evaporation of the ethereal extract gave a light brown oil, 183 mg., which crystallised from acetone to give cholesteryl bromide, m. p. and mixed m. p. 92—94°.

Acetolysis of Cholesteryl Bromide in the Presence of Acetic Anhydride.—An ampoule containing 500 mg. of cholesteryl bromide in 50 ml. of a 0·1M-solution of acetic anhydride in acetic acid was kept at 95° for 24 hr. Working up in the usual manner gave a brown oil (467 mg.). Chromatographic analysis on aluminium oxide gave 353 mg. of an oil from the pentane eluates and 35 mg. of an oil from the benzene–pentane eluates. Attempts to identify these products proved unsuccessful.

One of us (S. M.) acknowledges the financial support of the Swansea Education Authority; we thank Mr. J. R. Lewis, B.Sc., for assistance with the chromatographic analyses, and Glaxo Laboratories Ltd. for gifts of cholesterol.

UNIVERSITY COLLEGE OF SWANSEA, UNIVERSITY OF WALES. [*Received, August 10th,* 1954.]