solution was extracted with ether. The extract was washed to neutrality, dried over sodium sulfate and concentrated *in vacuo*. The residual oil (495 mg) was saponified in 30 ml of methanol to which 2 ml of 10% methanolic potassium hydroxide were added. After 15 hr at room temperature, the excess alkali was neutralized with acetic acid and the solution was diluted with water and extracted with ether. The ether solution was washed to neutrality, dried over sodium sulfate, and evaporated to yield a semicrystalline solid (487 mg). This product was chromatographed on 40 g of alumina using 200-ml fractions of benzene as eluent. Fractions 2–6 gave 300 mg of crystalline material, which was recrystallized several times from methylene chloride–chloroform to give IV: mp 135–137°, $[\alpha]^{24}D$ +65.2 ± 1° (28.2 mg in 2 ml of chloroform), $\lambda_{max}^{alcohol}$ 242 mμ (log ε 4.2); lit.[6] mp 137–138°, $[\alpha]D$ +66°.

Anal. Calcd for $C_{27}H_{44}O_2$: C, 80.93; H, 11.03. Found: C, 80.98; H, 11.19.

3β-Acetoxy-Δ⁵-etiocholenic acid was prepared from 3β,21-diacetoxy-Δ⁵-pregnen-20-one by periodate oxidation, using the procedure previously described:[18] mp 237–241°; lit.[18] 241–242°.

3β-Acetoxy-Δ⁵-etiocholenic acid chloride was prepared from 3β-acetoxy-Δ⁵-etiocholenic acid by the method of Steiger and Reichstein:[19] mp 157–159° and 300–310°; lit.[19] mp 331–332°.

21-Nor-20-ketocholesterol 3-acetate was prepared by treating 3β-acetoxy-Δ⁵-etiocholenic acid chloride with diisohexylcadmium as described by Kurath and Capezzuto:[10] mp 139–140°; lit.[10] mp 140–142°.

3β-Acetoxy-Δ⁵-cholesten-20β-ol (VI).—Twenty milliliters of a 3 *M* ether solution of methylmagnesium bromide was added dropwise (in an atmosphere of nitrogen) to an ice-cold solution of 3 g of 21-nor-20-ketocholesterol 3-acetate in 100 ml of dry benzene. The mixture was stirred for 1 hr in the cold and then overnight at room temperature. The ether was distilled and the remainder of the solution refluxed at 75° for 3 hr. After cooling, the Grignard complex was decomposed by ice-cold 10% sulfuric acid. The solution was extracted with ethyl acetate and the extract washed, dried over sodium sulfate, and evaporated *in vacuo*. The crude, oily residue (3.12 g) was acetylated in the usual way overnight and the oil obtained (3.22 g) was chromatographed on neutral alumina. The product isolated from the fractions eluted with ligroin B–benzene (6:4) was recrystallized from acetone–methanol to give 1.9 g (63.5%) of VI, mp 107–110°. Several recrystallizations from methanol gave a product of constant melting point (113–114°), $[\alpha]^{25}D$ −47 ± 3° (10.54 mg in 2 ml of chloroform).

Anal. Calcd for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 78.49; H, 10.87.

From the mother liquors, a crystalline product (0.6 g) was obtained, mp 124–127°. Fractional crystallization from ether gave two products, one melting at 131–138° and the other at 114–116°. Further crystallization of the higher melting compound yielded 145 mg (4.8%) of crystals, mp 151–155°. The infrared spectrum of this substance was identical with that of II. Further proof of identity was achieved by saponification and benzoylation to III.

20β-Hydroxycholesterol (V).—Compound VI (134 g, mp 111–112°) was saponified with methanolic KOH in the usual manner. The product was crystallized from methanol to afford 1.11 g of crystals, melting at 115–117°, $[\alpha]^{25}D$ −60.5 ± 3° (24.1 mg in 2 ml of chloroform). Admixture with the 20α-hydroxy isomer (I) gave a depression in melting point (97–115°).

3β-Benzoyloxy-Δ⁵-cholesten-20β-ol (VII).—Compound V (200 mg) was benzoylated as described previously. Crystallization of the product from ether–methanol gave needles: mp 144–145°, $[\alpha]^{25}D$ −33.9 ± 1° (20.6 mg in 2 ml of chloroform). Crystallization from acetone–methanol gave a sample consisting of broad plates. There was no depression in melting point on admixture of this compound with 20α-hydroxycholesterol 3β-benzoate (III).

Anal. Calcd for $C_{34}H_{50}O_3$: C, 80.58; H, 9.94. Found: C, 80.47; H, 9.98.

20β-Hydroxy-Δ⁴-cholesten-3-one (VIII).—Compound V (200 mg) was oxidized by Jones solution as described for the 20α-hydroxy isomer. Chromatography of the crude product on alumina gave material which crystallized from acetone–hexane mp 129–130°, $[\alpha]^{24}D$ +76.6° ± 2° (15.65 mg in 2 ml of chloroform), $\lambda_{max}^{alcohol}$ 242 mμ (log ε 4.19). When this compound was mixed with IV, a large depression (110–124°) in melting point resulted.

Anal. Calcd for $C_{27}H_{44}O_2$: C, 80.93; H, 11.03. Found: C, 80.92; H, 11.10.

Registry No.—I, 516-72-3; IV, 384-27-0; VI, 7429-99-4; VII, 7445-08-1; VIII, 7430-00-4; 3β-acetoxy-Δ⁵-etiocholenic acid, 7150-18-7; 21-nor-20-ketocholesterol 3-acetate, 6570-97-4.

(18) P. Hegner and T. Reichstein, *Helv. Chim. Acta*, **24**, 828 (1941).
(19) M. Steiger and T. Reichstein, *ibid.*, **20**, 1164 (1937).

On Cyclic Intermediates in Hydrolytic Reactions. II. Solvent and Salt Effect in the Alkaline Hydrolysis of Dinitro-2-pyridylalanylglycine

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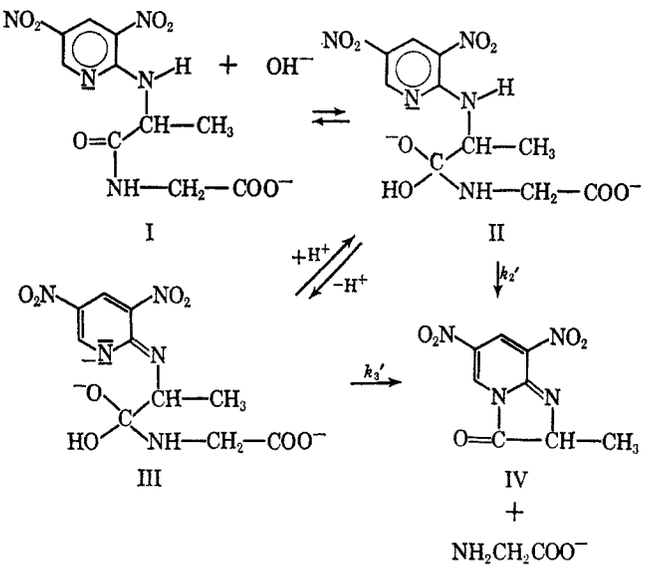
The alkaline hydrolysis of dinitro-2-pyridylalanylglycine (I) has been studied previously;[1] this earlier investigation was concerned mainly with the mechanism of hydrolysis in aqueous solution and the results were consistent with a reaction sequence in which both neutral pyridine nitrogen and pyridyl mesomeric anion give a nucleophilic displacement of glycine anion through neighboring group catalysis (see Scheme I).

SCHEME I



The kinetics of hydrolysis were followed by measurements of the appearance of glycine; moreover it was possible to demonstrate the formation and decomposition of 6,8-dinitroimidazo[1,2a]pyridin-3(2H)-one (IV) in this hydrolysis and to measure its rate of hydrolytic cleavage. The following rate equation was derived on

$$k_1 = k_2[OH^-] + k_3[OH^-]^2 \quad (1)$$

the basis of the proposed reaction scheme where k_1 is the observed first-order rate constant.

(1) A. Signor, L. Biondi, and E. Bordignon, *J. Org. Chem.*, **31**, 1403 (1966).

It was considered, however, desirable to obtain further information concerning the dianion intermediate III which is required to explain the third-order kinetics observed. There are several examples in the literature of analogous intermediates, namely in the aqueous alkaline cleavage of acetylacetone[2] and in the Cannizzaro reaction;[3] a further analogy is provided by the alkaline hydrolysis of N-methylanilides.[4] In recent years there has been increasing interest in the catalysis of reactions by metal ions and in the large specific kinetic salt effects which sometimes occur.[5-7] Metal ions have been shown to catalyze a large number of organic reactions; the alkaline hydrolysis of potassium ethyl oxalate and potassium ethyl malonate is catalyzed by calcium ion, barium ion, and thallous ion in that order. Alkali metal ions, on the other hand, have only a small negative salt effect on the hydrolysis of potassium ethyl malonate. On the basis of the structural and metal–ion effects, it was postulated[8] that the transition states of the hydrolyses, which are catalyzed by calcium, barium, and thallous ions, can be represented by a chelate structure; the transition state is thereby stabilized and the energy barrier is lowered.

Therefore it seemed that the rates of alkaline hydrolysis of I in the presence of various cations might throw further light on the proposed mechanism.

The purpose of the present study was to reinvestigate the reaction in order (1) to obtain the various thermodynamic quantities of activation, (2) to determine the influence of the dielectric constant of the solvent upon the rate, and (3) to test the applicability of the theories dealing with electrostatic effects on ionic reaction rates.

Experimental Section

Materials.—Dinitro-2-pyridylalanylglycine has been prepared by the method described in a precedent paper;[9] recrystallization from water–ethanol affords a chromatographically pure product, mp 180°. The sodium, lithium, and barium chloride used were C. Erba products, reagent grade.

Carbonate-free sodium and lithium hydroxide solutions were prepared by dilution of concentrated stock solutions prepared by dissolving chemically pure sodium or lithium hydroxide in water in 1:1 weight ratio. The concentrated solution was allowed to stand in a covered container for 24 hr, after which the clear supernatant liquid was decanted and filtered in order to remove carbonate crystals.

Standardization of Barium Hydroxide.—Solutions of reagent grade barium hydroxide dissolved in distilled water which has been boiled and cooled under nitrogen were centrifuged to remove any precipitated barium carbonate and the barium was determined volumetrically.

Kinetic Runs.—The hydrolysis experiments were performed in a thermostated water bath whose temperature was held constant to ±0.1°. All reactions were run at 1.00 *M* ionic strength, unless otherwise stated; the mechanical procedures employed in performing the rate determinations are described in earlier paper.[1]

Results and Discussion

Temperature Dependence.—In order to determine the activation parameters of the alkaline hydrolysis of dinitro-2-pyridylalanylglycine we must take separately into account the effect of temperature on second- and third-order rate constants; we have performed rate measurements at several temperatures in the range 21–48° and plotted $k_1/[OH^-]$ against $[OH^-]$. In this way it is possible to obtain from intercept and slope the relative values of k_2 and k_3 at each temperature. In Table I are reported the experimental results.

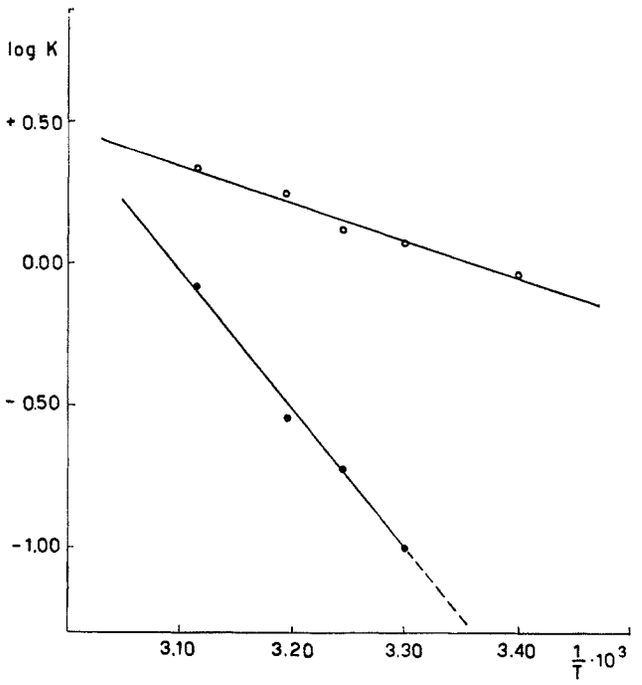


Figure 1.—Arrhenius plot between 20 and 48° for second-order constant (●) and third-order constant (○) in the alkaline hydrolysis of dinitro-2-pyridylalanylglycine.

An Arrhenius plot was constructed from the data of Table I; Figure 1 shows that the activation energies are reasonably constant over the temperature range studied. The slopes of the lines, computed from all the points by the method of least squares, correspond to an activation energy of 23.4 kcal/mole for the second-order process and 6.4 kcal/mole for the third-order process; the value of the frequency factor A (in the relation $k = Ae^{-E/RT}$) for the third-order process, likewise computed from all the points, is 8.1×10^2 $l.^2$ $mole^{-2}$ sec^{-1} ($\Delta S^{\pm} = -47.2$ eu). It is well known that for reactions between ions of like sign there is an entropy decrease going from reactants to activated complex; in terms of solvation the explanation is that for ions of the same sign the transition state will be a more highly charged ion which would be expected to be strongly solvated, so that more solvent molecules might be required than for the separate ions.

Solvent Effect.—An exact prediction on the effect of different solvents on ionic equilibria is not possible. However, certain generalizations can be made from a simple electrostatic picture; the elementary electrostatic theory will be useful in giving the general effect of the charges on the ionic reactants and the dielectric constant of the medium. It is possible to write an

(2) R. G. Pearson and E. A. Mayerle, *J. Am. Chem. Soc.*, **73**, 926 (1951).
(3) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p 350.
(4) S. S. Biechler and R. W. Taft, Jr., *J. Am. Chem. Soc.*, **79**, 4927 (1957).
(5) M. Kilpatrick, *Ann. Rev. Phys. Chem.*, **2**, 269 (1951).
(6) A. Indelli, *Trans. Faraday Soc.*, **59**, 1827 (1963).
(7) E. W. Westhead and H. Morawetz, *J. Am. Chem. Soc.*, **80**, 237 (1958).
(8) J. I. Hoppe' and J. E. Prue, *J. Chem. Soc.*, 1775 (1957).
(9) A. Signor, L. Biondi, M. Terbojevich, and P. Pajetta, *Gazz. Chim. Ital.*, **94**, 619 (1964).

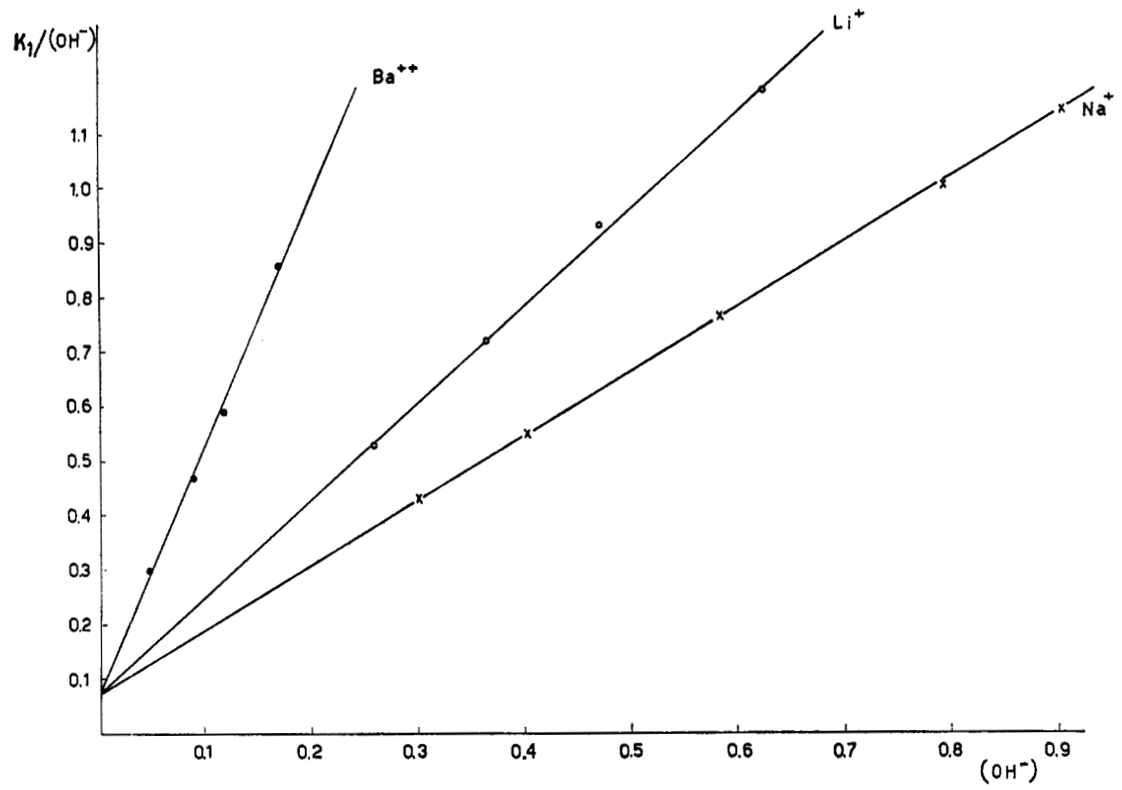


Figure 2.—Influence of some metal ions on the rate constants of alkaline hydrolysis of dinitro-2-pyridylalanylglycine; ionic strength constant 1.00 M.

TABLE I

RATE CONSTANTS OF ALKALINE HYDROLYSIS OF DINITRO-2-PYRIDYLALANYLGLYCINE AT VARIOUS TEMPERATURES[a]

$[OH^-]$, M	k_1, min^{-1}	$k_1/[OH^-]$	k_1, calcd
	Temperature 21°		
0.306	0.080	0.261	0.093
0.416	0.160	0.385	0.173
0.621	0.370	0.596	0.385
0.810	0.630	0.778	0.655
	Temperature 30°		
0.404	0.220	0.550	0.234
0.489	0.360	0.735	0.333
0.581	0.445	0.768	0.458
0.795	0.860	1.080	0.811
0.905	1.040	1.150	1.055
	Temperature 35°		
0.245	0.120	0.490	0.120
0.355	0.220	0.619	0.217
0.455	0.345	0.759	0.341
0.552	0.470	0.851	0.480
0.722	0.780	1.080	0.777
	Temperature 40°		
0.148	0.075	0.520	0.080
0.239	0.165	0.690	0.168
0.351	0.325	0.930	0.312
0.416	0.442	1.060	0.421
0.550	0.690	1.250	0.686
0.580	0.730	1.260	0.754
	Temperature 48°		
0.146	0.170	1.160	0.160
0.245	0.345	1.410	0.331
0.356	0.560	1.580	0.562
0.490	0.950	1.940	0.933

[a] NaCl was added to make ionic strength 1.00 M.

expression (which is shown below) for the dependence of the rate constant on the dielectric constant[10]

$$\ln k = \ln k_0' - \frac{NZ_AZ_Be^2}{DRTr^{\ddagger}} \quad (2)$$

where k_0' is the specific rate constant in a medium of infinite dielectric constant. This equation predicts a linear plot of log k against $1/D$ with a negative slope if the charges of the ions are of the same sign and a positive slope if the charges are of opposite sign. In agreement with the predictions of the theory, the slope of log k *vs.* $1/D$ for the alkaline hydrolysis of dinitro-2-pyridylalanylglycine is negative; the experimental data for some water–ethanol mixtures are reported in Table II. For $D < 65$ the curve exhibits a rapid change of slope comparable with that observed by Amis and La Mer for the bromophenol blue–hydroxide ion reaction.[11]

TABLE II

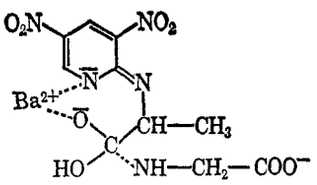
DINITRO-2-PYRIDYLALANYLGLYCINE PLUS HYDROXIDE ION IN WATER–ETHANOL AT 30°

Wt % of ethanol	D	$1/D$	k_1, min^{-1}	Log k_1
0.00	78.5	0.0127	0.625	−0.204
8.24	73.7	0.0136	0.502	−0.299
12.07	71.4	0.0140	0.480	−0.319
17.23	68.4	0.0146	0.425	−0.372

Catalysis by Barium and Alkali Metal Ions.—In order to determine the effect of barium ions on the rate of alkaline hydrolysis of I, a study was made in the presence of varying concentrations of barium hydroxide; barium ions exert a powerful catalytic effect and

(10) G. Scatchard, *Chem. Rev.*, **10**, 229 (1932).
(11) E. S. Amis and V. K. La Mer, *J. Am. Chem. Soc.*, **61**, 905 (1939).

we have observed a great enhancement in the rate constants of hydrolysis. The plot of $k_1/[OH^-]$ against $[OH^-]$ is strictly linear (Figure 2); it can be seen that the bivalent heavy metal ion has no influence on k_2, but increases markedly the value of k_3, the rate constant of the intramolecular process which requires a dianion intermediate in the proposed reaction scheme. We regard this as a catalytic effect arising from the formation of chelate complex between the metal ion and the transition state. A similar stabilization of the transition state by chelate formation is probably responsible for the catalysis by heavy metal ions of the hydrolysis of amino acid esters;[12] if our interpretation is correct, it follows that the transition state contains a barium ion and can be represented by chelate structure V.



V

Alkali metal ions, on the other hand, might be expected to display a more general type of catalytic effect owing to their electrophilic powers which are in many ways analogous to those of the proton. The rates of alkaline hydrolysis of dinitro-2-pyridylalanylglycine have been studied in the presence of sodium and lithium chloride; as can be seen from Figure 2 there is a rather large salt effect of the alkali metal ion on the hydrolysis. Furthermore a few measurements performed as a function of ionic strength have evidentiated a negative specific salt effect probably owing to the separation of the charge of the pyridyl group from the reaction center. This is in agreement with the postulated dianion intermediate which will behave as a conventional doubly charged ion in sufficiently dilute solution; when the radius of the Debye–Hückel ionic atmosphere becomes comparable with the separation between the charges, each charge will tend to build up its own ion atmosphere and simulate independent ions.

Registry No.—I, 2900-34-7.

(12) K. Kroll, *J. Am. Chem. Soc.*, **74**, 2036 (1952).

The Evidence Against Acetyl Migration during the Acetylation of Methyl Cholate

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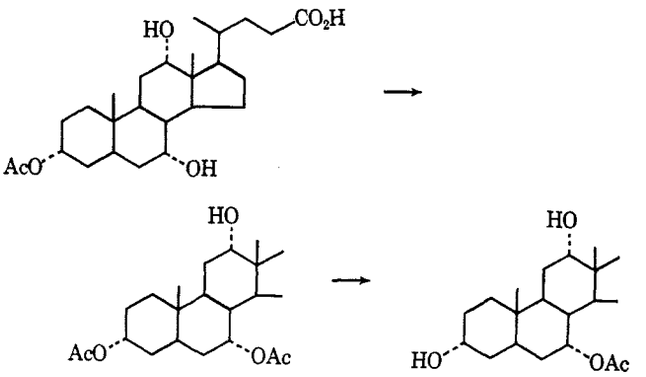
The order of reactivity of hydroxyl groups of methyl cholate toward acetylation is described as 3 > 7 > 12, and is based on formation of the 3-monoacetate[1] and the 3,7-diacetate.[2] Fieser and Fieser view acetyl migration from 3 to 7 during formation of the diacetate as unlikely on the basis of the indirect evidence that the cathylate group does not migrate.[3] Because of our recent finding[4] that with derivatives of methyl cholanate the 12α-hydroxyl group is inherently more reactive toward acetic anhydride than the 7α-hydroxyl group, we sought direct evidence on the question of acetyl migration *via* stepwise synthesis, then selective hydrolysis of the 3,7-diacetate.



Methyl cholate 3-acetate, prepared with 1-C^{14} acetic anhydride, was further acetylated with unlabeled anhydride. The 3,7-diacetate was selectively hydrolyzed with methanolic HCl to the 7-monoacetate.[5] If the labeled acetyl group had migrated to the 7-oxygen in the preparation of the diacetate, it would have been retained in the final step; loss of more than 99% of the radioactivity rules out any significant amount of 3→7 migration This result is confirmed in the second experiment where the order is reversed and nearly all of the radioactivity was retained. This work fully corroborates the Fieser postulate.

We are currently studying side-chain shielding[6] and other explanations for 3,7- rather than 3,12-diacetate formation.[4]

Experimental Section

Melting points are uncorrected. The infrared spectra were taken on mulled samples with a Perkin-Elmer Infracord. Liquid scintillation counting was carried out in a Packard Tri-Carb.

Synthesis and Hydrolysis of Methyl Cholate 3-(1-C^{14} Acetate) 7-Acetate.—A solution of 1.26 g of methyl cholate in 7 ml of benzene was distilled until 3 ml had been removed. To this solution at reflux was added a solution of 0.94 ml of 1-C^{14} acetic anhydride (prepared by diluting 0.5 mcurie of Ac_2O, specific activity 4 mcuries/mmole, with 4.5 ml of cold reagent and distilling at atmospheric pressure) in 1.4 ml of benzene. The solution was refluxed for 2 hr, then evaporated in an open dish. The residual oil crystallized from acetone–ether to give a crude product (618 mg), mp 137–141°. Recrystallization in acetone–ether and acetone–petroleum ether (bp 30–60°) raised the melting point to 144–146°. Two more recrystallizations from methanol–water to constant specific activity gave the 3-monoacetate: 203 mg; mp 146.5–148° (lit.[1] mp 149–150°); λ_{max} 2.83 (sh), 2.97 (OH), 5.74 μ (C=O), 4324 × 10^6 cpm/mole.

Unlabeled acetic anhydride (0.12 ml) was added to a solution of 192 mg of labeled 3-monoacetate in 1 ml of benzene and 0.12 ml of pyridine. After 24 hr at room temperature, this solution was poured into 10 ml of water and 6 ml of ether. The organic

(1) R. Grand and T. Reichstein, *Helv. Chim. Acta*, **28**, 347 (1945).

(2) L. F. Fieser, S. Rajagopalan, E. Wilson, and M. Tishler, *J. Am. Chem. Soc.*, **73**, 4133 (1951).

(3) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p 221.

(4) To be reported elsewhere.

(5) L. F. Fieser and S. Rajagoplan, *J. Am. Chem. Soc.*, **72**, 5530 (1950). Methanol containing about 1% concentrated HCl was found by Dr. Amira Sattar of these laboratories to be fully as satisfactory and more convenient than Fieser and Rajagopalan's solution of anhydrous HCl.

(6) Reference 3, p 222.