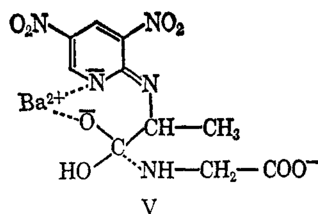
we have observed a great enhancement in the rate constants of hydrolysis. The plot of $k_1/[OH^-]$ against $[OH^-]$ is strictly linear (Figure 2); it can be seen that the bivalent heavy metal ion has no influence on k_2, but increases markedly the value of k_3, the rate constant of the intramolecular 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.



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.

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The Evidence Against Acetyl Migration during the Acetylation of Methyl Cholate

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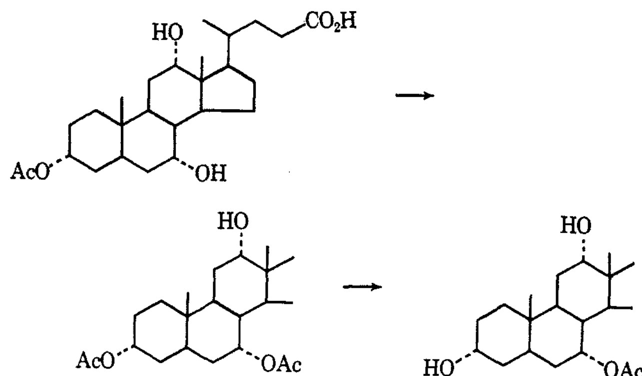
The order of reactivity of hydroxyl groups of methyl cholate toward acetylation is described as $3 > 7 > 12$, and is based on formation of the 3-monoacetate[1] and the 3,7-diacetate.[2] Fieser and Fieser view acetyl migration from 3 to 7 during formation of the diacetate as unlikely on the basis of the 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 Rajagoplan's solution of anhydrous HCl.

(6) Reference 3, p 222.

layer was washed with water and evaporated; recrystallization of the solid residue out of methanol–water gave crude diacetate, 153 mg, mp 169–180°. Two recrystallizations to constant specific activity using acetone–water gave feathery needles: mp 185–186° (lit.[2] mp 187–188°); λ_{max} 2.79 (OH), 5.75, 5.83 μ (C=O), 4368 × 10^6 cpm/mole.

A solution of 67 mg of the diacetate in 0.87 ml of methanol and 0.01 ml of concentrated HCl was stirred for 30 hr at room temperature. Water was added and the turbid mixture was cooled and filtered. A benzene solution of the crude 7-monoacetate was allowed to evaporate overnight, leaving fine needles, mp 177–178.5°. It was recrystallized to constant specific activity by two recrystallizations in benzene–petroleum ether; the pure product (29 mg) showed mp 179–179.5° (lit.[5] mp 178–179°); λ_{max} 2.83 (OH), 5.79 μ (C=O), 29 × 10^6 cpm/mole.

Synthesis and Hydrolysis of Methyl Cholate 3-Acetate 7-(1-C^{14} Acetate).—Using the labeled acetic anhydride mentioned above, methyl cholate 3-acetate in benzene and pyridine was converted to the diacetate and recrystallized to constant specific activity; mp 186–188°, 4298 × 10^6 cpm/mole. This was hydrolyzed in methanolic HCl to the 7-monoacetate and recrystallized to constant specific activity, mp 177–179°, 4291 × 10^6 cpm/mole.

Registry No.—3-Labeled diacetate, 7432-66-8; methyl cholate, 1448-36-8; 7-labeled diacetate, 7432-68-0; 7-labeled acetate, 7432-69-1; 3-labeled acetate, 7430-18-4.

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A Convenient Synthesis of Arabinosylcytosine (Cytosine Arabinoside)[1,2]

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In the original preparation of arabinosylcytosine (1-β-D-arabinofuranosyl cytosine),[3] polyphosphoric acid was used to convert 2′(3′)-cytidylic acid to a phosphorylated 2,2′-anhydrocytidine and the latter was subsequently hydrolyzed and dephosphorylated to give the cytidine epimer. Ion-exchange fractionation at the nucleotide stage was required to separate arabinosylcytosine 3′,5′-diphosphate from cytidine derivatives. An alternative multistep synthesis was later described by Evans, *et al.*,[4] involving the conversion of uridine to 1-β-D-arabinofuranosyluracil *via* the 2,2′-anhydride[5] with eventual conversion to the corresponding cytosine derivative by thiation and ammonation.[6] Shen, *et al.*,[7] have described a more practical synthesis which utilizes the readily available 2,3,5,-tri-O-benzyl-D-arabinosyl chloride of Glaudemans and Fletcher.[8] Since completion of the present study several new approaches to synthesis have been reported.[9,10]

The availability of this nucleoside analog has made possible certain metabolic experiments[11] as well as tests to establish its chemotherapeutic value as an antitumor and antiviral agent.[12–14] The initial success of such tests, particularly those directed toward DNA-containing viruses, has led to the demand for large quantities of the arabinosyl derivative for clinical testing. A procedure based on a new method of nucleoside fractionation[15] which can be conveniently applied to the preparation of gram or kilogram quantities is herein described.

Experimental Section

Cytidine (5 g) or the molar equivalent of 2′(3′)-cytidylic acid is suspended in 100 g of polyphosphoric acid in a stoppered flask[16] and the suspension is heated at 80° for 30 hr.[17] The dark brown, homogeneous mass is dissolved in 200 ml of water and the solution is heated at 100° for 60 min to break pyrophosphate bonds. The solution is brought to pH 9 by the slow addition of 10% lithium hydroxide resulting in hydrolysis of the 2,2′-anhydro intermediate and giving, as the major product, the 3′,5′-diphosphate of arabinosylcytosine.

To the filtrate remaining after removal of lithium phosphate is added 20 g of magnesium chloride, 20 ml of 30% ammonium chloride, and concentrated ammonium hydroxide to pH 9.5. The magnesium ammonium phosphate is removed by filtration, 20 mg of alkaline phosphatase[18] is added to the filtrate, and the solution is incubated under toluene at 37° for 24 hr. Paper chromatography in isopropyl alcohol–ammonia–water (7:1:2) can be used to demonstrate complete conversion of nucleoside mono-, di-, and triphosphates to free nucleosides. The magnesium ammonium phosphate is then removed and the filtrate (*ca.* 1500 ml) is concentrated to 200 ml to remove excess ammonia. After dilution to 400 ml with water, the solution is placed on a 4.7 × 15 cm column of Dowex 50-8x (H^+, 50–100 mesh). Elution with water (1500 ml) yields the mineral acids derived from the anions of the salts plus uracil nucleosides (60 mg) resulting from slight deamination of the corresponding cytosine compounds. Cytidine and arabinosylcytosine are then displaced with 1 *N* ammonium hydroxide (1000 ml). The fractions containing the pentofuranosyl derivatives of cytosine are evaporated to dryness to remove ammonia, taken up in 35 ml of 30% methanol, and applied to a 3.6 × 13 cm column of Dowex 1-2x (OH^-, 200–400 mesh) previously equilibrated with 30% methanol. Upon elution with 30% methanol, cytidine emerges and can be recovered in chromatographically pure form by evaporation of solvent (recovery 423 mg).

After 1500 ml of aqueous methanol has passed through the column, it is stripped with 0.1 *M* ammonium bicarbonate. The arabinosylcytosine appears coincident with the bicarbonate front and is completely eluted in a volume of *ca.* 200 ml. Repeated flash evaporation of the aqueous solution decomposes the

(1) This work was supported by a grant (GB-882) from the National Science Foundation.

(2) In keeping with the "Rules of Carbohydrate Nomenclature" [*Chem. Eng. News*, **31** (17), 1776 (1953)], the term "arabinosylcytosine" has been used throughout this manuscript instead of the commonly applied "cytosine arabinoside." The latter term has been retained in the title for the convenience of "keyword index" users.

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(14) D. A. Buthala, *Proc. Soc. Exptl. Biol. Med.*, **115**, 69 (1964).

(15) C. A. Dekker, *J. Am. Chem. Soc.*, **87**, 4027 (1965).

(16) Prewarming the polyphosphoric acid reduces its viscosity and facilitates handling.

(17) The progress of the reaction can be estimated by dilution of a small aliquot of the reaction mixture with cold 0.1 *N* hydrochloric acid and examination of the OD_{280}/OD_{260} ratio. When the value has decreased to *ca.* 0.7, extensive formation of the 2,2′-anhydride intermediate has occurred.

(18) Worthington Biochemical Corp. preparation PC-P 639.