Anal. Calcd. for $C_{39}H_{54}O_3N_8$: N, 14.69. Found: N, 15.24.

Cholestenone from III.—III (50 mg.), refluxed for one hour in 4% methanolic potassium hydroxide (15 ml.), gave a neutral fraction (42 mg.), which crystallized to yield cholestenone, m.p. 78–80°.

Cholestenone-4-C^{14}.—$C^{14}H_3I$ (originally 1.8 millicuries in 266 mg.), which had been stored in the dark for four years but which was deeply pigmented, was distilled through drierite (clear distillate). The system was then flushed with an equal weight of carrier methyl iodide. The Grignard reagent prepared from the combined alkyl halide (3.74 millimoles) reacted, as described above, with 1.2 g. (3.1 millimoles) of the enol-lactone I to furnish, on direct crystallization, 650 mg. (52%) of the hemiacetal II, m.p. 160–175°, count 6.12×10^8. The mother liquors yielded 270 mg. of 3,5-seco-5-keto-cholestan-3-oic acid, m.p. and admixture m.p. 151–154°. This recovery of starting material, not encountered in C^{12} runs with the same molar proportion of methyl iodide, may be due to (a) impurities in the small sample of $C^{14}H_3I$, (b) loss of appreciable total alkyl halide through decomposition on long storage, (c) difference in reaction rate between C^{12} and C^{14}, or a combination of the three factors.

II (650 mg.) was converted by procedure A above to 526 mg. (1.37 millimoles, 45%) of cholestenone-4-C^{14}, m.p. 76–78°, count 6.12×10^8.

McGILL UNIVERSITY
MONTREAL, CANADA

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Preparation of Acetic-2-C^{14} Acid[1]

BY DANIEL N. HESS

A method has been developed for the preparation of high specific activity acetic-2-C^{14} acid from methanol-C^{14}. The method, which involves the intermediate formation of methyl hydrogen sulfate and acetonitrile, is simpler and is better suited to the synthesis of a product with high specific activity than the conventional method involving carbonation of methyl-C^{14}-magnesium iodide.[2] Yields averaging 87% have been obtained on a 10-millimole scale.

Experimental

A 10.5-millimole portion of crystalline sulfur trioxide was introduced into the reaction flask[3] equipped with a magnetic stirring bar. The flask was quickly attached to a vacuum line, and the 10-ml. bulb was immersed in liquid nitrogen. A 10.06-millimole aliquot of methanol vapor (39.2 millicuries), measured manometrically, was added to the reaction flask. The liquid nitrogen bath was replaced by an ice-bath, and the reaction mixture was stirred. After the initial reaction had subsided, an additional one-half hour at room temperature was allowed for completion of the reaction. Complete reaction was demonstrated by the absence of methanol vapor pressure as determined with a McLeod gage.

The flask was removed from the vacuum line, the bulb was immersed in liquid nitrogen, and 10 ml. of 7.5 *M* potassium cyanide was added dropwise. After the flask was allowed to warm slowly to room temperature with stirring for one-half hour, the acetonitrile solution was distilled into a calibrated 40-ml. flask. Three successive 10-ml. portions of water were added to the reaction flask and distilled to ensure complete transfer of the acetonitrile. Radioactivity assay showed a 96% yield to acetonitrile-2-C^{14}.

The acetonitrile was hydrolyzed by refluxing with 50 millimoles of potassium hydroxide for 24 hours. The radiochemical yield to potassium acetate-2-C^{14}, based on methanol, was 91%.

The alkaline solution of potassium acetate was acidified with 85% phosphoric acid and titrated with a solution of potassium permanganate.[4] The solution was distilled to dryness after the addition of three successive small portions of water. The distillate was titrated with potassium hydroxide, and the water evaporated. The potassium acetate was dried at high vacuum until no pressure greater than 10^{-4} mm. was observed after standing one-half hour under static vacuum at 120°.

The dried salt was covered with phosphoric acid thoroughly saturated with phosphorus pentoxide, the flask was attached to the vacuum line, and the acetic acid was collected in a liquid nitrogen cooled receiver. When the rate of evolution subsided, the flask was gradually heated to 120°, and the temperature maintained until there was no further evolution of acetic-2-C^{14} acid. Thirty-five and two-tenths millicuries of acetic-2-C^{14} acid was obtained, or a yield of 90% based on methanol-C^{14}. In order to determine the purity of the acetic acid prepared by this procedure, the product from a typical run and a purified derivative were analyzed using dilution technique. The radioactivity of the diluted acetic acid was 2.49 μc. per mmole, and the radioactivity of the derivative was 2.52 μc. per mmole. Thus, the product is pure within the limits of the analytical method which has an estimated error of ±1%.

Carbon-14 analyses of the methanol, acetonitrile and acetic acid were made on the methyl-3,5-dinitrobenzoate, phloroacetophenone and *p*-nitrobenzyl acetate derivatives, respectively. These derivatives prepared from appropriately diluted samples were converted to carbon dioxide by wet-combustion and assayed for radioactivity by determination of the ion current with a dynamic condenser electrometer. The isotopic dilution method of determining yields[5] and the carbon-14 analysis procedure[6] have been published.

Acknowledgment.—The author wishes to express his indebtedness to Dr. O. K. Neville for his advice and interest in the execution of this project.

(4) This destroyed any cyanide and formate that might be present.

(5) G. A. Ropp, THIS JOURNAL, **72**, 4459 (1950).

(6) O. K. Neville, *ibid.*, **70**, 3499 (1948).

CHEMISTRY DIVISION
OAK RIDGE NATIONAL LABORATORY
OAK RIDGE, TENN.

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Improvements in the Preparation of L-Arabinose from Mesquite Gum[1]

BY C. S. HUDSON

Directions for the preparation of L-arabinose from mesquite gum have been published by Anderson and Sands,[2] by Isbell[3] and recently by White,[4] who has made radical improvements on the older directions. Present knowledge of the structure of mesquite gum, recently reviewed by Jones and Smith,[5] indicates that its graded acid hydrolysis can be expected to liberate principally the L-arabinose moiety as the first step. However, in the earlier methods[2,3] experience showed that it was necessary to continue the acid hydrolysis considerably beyond this first stage in order to be able to control the foaming during subsequent operations. Our experience with the earlier methods[2,3] was not encour-

(1) This document is based upon work performed under Contract Number W-7405, eng. 26 for the Atomic Energy Project at Oak Ridge National Laboratory.

(2) B. M. Tolbert, *J. Biol. Chem.*, **173**, 205 (1948).

(3) The reaction flask consisted of a 25-ml. flask with a 10-ml. bulb sealed onto the bottom. The purpose of the bulb was to contain the small quantities of reagents in the sulfonation phase of the experiment. The larger bulb provided ample volume for subsequent reactions.

(1) Presented at the Portland, Oregon, Meeting of the American Chemical Society in September, 1948.

(2) E. Anderson and Lila Sands, (a) THIS JOURNAL, **48**, 3172 (1926); (b) *Org. Syntheses*, **8**, 18 (1928).

(3) H. S. Isbell in "Polarimetry, Saccharimetry and the Sugars," Circular C440, Natl. Bur. Standards, p. 457 (1942).

(4) E. V. White, THIS JOURNAL, **69**, 622, 715 (1947).

(5) J. K. N. Jones and F. Smith, *Advances in Carbohydrate Chem.*, **4**, 243 (1949).

aging because the purification of the resulting crude L-arabinose was very difficult.[6]

White[4] has been able to reduce the degree of acid hydrolysis of mesquite gum approximately to the ideal stage where the solution contains most of the L-arabinose as free sugar accompanied by residual degraded gum of high molecular weight, and he has then employed dialysis to separate these components.

The new information which is now presented permits the use of White's low acid hydrolysis proce dure without the subsequent employment of dialysis. It has been found that if the original mesquite gum solution is boiled a few hours in alkaline reaction ($Ba(OH)_2$) the subsequent step of ideal mild acid hydrolysis can then be combined with the separation and isolation of the L-arabinose by the customary procedures of the earlier methods,[2,3] thus avoiding dialysis. An explanation of the fact that the preliminary boiling of the mesquite–barium hydroxide solution overcomes in large measure the troublesome foaming characteristics is not known, but the observation may be of significance for future studies of gums and mucilages.

Directions for the Preparation of L-Arabinose from Mesquite Gum.—Place in a four-liter erlenmeyer flask 260 g. of mesquite gum, 13 g. of barium hydroxide octahydrate and 1500 ml. of water. Heat the mixture on the steam-bath one to two hours with occasional agitation. Then reflux the solution over a flame for two hours, taking care in the early period that it does not foam into the condenser; it soon boils with little foaming. When this alkaline treatment is completed, add 360 ml. of *N* sulfuric acid; the barium is thus precipitated as sulfate and the solution becomes about 0.14 *N* through the excess of sulfuric acid. Since mesquite gum yields a small amount of ash this calculated normality is doubtless higher than the effective acidity. This normality is approximately that used by White and it is less than one-tenth of the acidity used by Anderson and Sands or by Isbell. Without filtration, the solution is refluxed for four hours; at this low acidity the partial hydrolysis of the gum during boiling liberates most of the L-arabinose without producing other simple carbohydrates in amounts that would seriously impede the crystallization of the pentose. Decolorizing carbon (25 g.) is then added and the solution is neutralized with an excess of calcium carbonate (40 g.), which is added slowly to control foaming; neutralization is completed (litmus test) by heating on the steam-bath one or two hours. Ten grams of filtercel is added, the solution is filtered on a Buchner funnel and the cake is washed with 400 ml. of hot water. The filtrate and washings are concentrated under reduced pressure to a volume of about 225 ml.; ordinarily there is little foaming during this concentration but in any event it may be controlled by adding a few drops of octyl alcohol. (Without the preliminary boiling of the gum solution with barium hydroxide, the concentrating of the acid-hydrolyzed solution under reduced pressure is most troublesome and sometimes impossible because of foaming.) Warm methanol (about 120 ml.) is then mixed with the sirup and the solution is poured with good stirring into 1500 ml. of warm methanol in a four-liter beaker kept on the steam-bath. There is an abundant precipitation of calcium salts in nicely granular condition. The clear liquor is decanted, the salts are ground in a mortar, returned with the liquor to the beaker, the distilling flask and mortar are washed with about 350 ml. of methanol and the salts are digested warm a few minutes with the total methanol extract. The mixture is filtered after the addition of 15 g. of decolorizing carbon and 10 g. of filtercel and the cake is washed with 300 ml. of warm methanol. The clear methanol solution and washings are concentrated under reduced pressure to about 150 ml. L-Arabinose often crystallizes in part during this concentration; at the end it is dissolved by warming the sirup, which is then poured from the flask and diluted with 25 ml. of methanol that is used to clean the flask. This solution is allowed to crystallize one day at room temperature, followed by several days in a refrigerator. The L-arabinose is removed by filtration and washing with cold methanol. The sugar weighs about 60–70 g., and its equilibrium rotation in water is about $[\alpha]^{20}D$ +100–101°. It may be recrystallized from about 70% of its weight of water. At least one recrystallization from water is advisable; the rotation of the product is then usually very near the +104° value of pure L-arabinose. Methanol may be used for subsequent recrystallizations if they are required. The yield may be increased somewhat by allowing the original crystallization to proceed a longer time near 0°; yields of 80–90 g. have been obtained in this way with solutions that were preserved several months. Although the use of ion-exchange materials is not included in the directions, experiments have indicated their usefulness in removing inorganic matter from impure arabinose.[7]

(6) R. C. Hockett and C. S. Hudson, THIS JOURNAL, **56**, 1632 (1934).

(7) H. G. Fletcher, Jr., H. W. Diehl and C. S. Hudson, *ibid.*, **72**, 4546 (1950).

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE
FEDERAL SECURITY AGENCY
BETHESDA, MARYLAND

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The Reaction of Chloramphenicol (Chloromycetin[1]) with α-Chymotrypsin

BY H. T. HUANG AND CARL NIEMANN[2]

The observation of Smith and Worrel[3] that chloramphenicol, *i.e.*, D(−)-*threo*-1-*p*-nitrophenyl-2-dichloroacetamido-1,3-propanediol, in concentrations up to 0.69×10^{-3} *M* has no measurable effect upon the α-chymotrypsin-catalyzed hydrolysis of casein, under the conditions specified in the assay method of Anson and Mirsky[4] has led us to investigate the inhibitory properties of chloramphenicol under more favorable conditions since it is known that the degree of competitive inhibition by a given inhibitor is not only determined by the affinity constant of the enzyme and inhibitor but also by that of the enzyme and specific substrate and by the relative molar concentrations of enzyme, substrate and inhibitor. We have examined the effect of chloramphenicol upon the α-chymotrypsin-catalyzed hydrolysis of nicotinyl-L-tryptophanamide and acetyl-L-tyrosinamide at 25° and *p*H 7.9 in aqueous solutions 0.02 *M* in respect to the amine component of a tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer. The analytical methods were identical with those described previously.[5,6] The results of the main series of experiments, wherein nicotinyl-L-tryptophanamide was used as the specific substrate, are summarized in Fig. 1. From the nature of the conventional $1/v_0$ *versus* $1/[S]_0$ plot[7] and the knowledge that K_S for the system α-chymotrypsin-nicotinyl-L-tryptophanamide, under the conditions specified, is 2.7×10^{-3} *M*[5] it may be concluded that chloramphenicol functions as a competitive inhibitor in the above system and that K_I, the enzyme-inhibitor dissociation constant, at 25° and *p*H 7.9, is 13.5×10^{-3} *M*. Duplicate experiments with acetyl-L-tyrosinamide,

(1) Parke Davis and Co. trade mark.

(2) To whom inquiries regarding this article should be sent.

(3) G. W. Smith and C. S. Worrel, *Arch. Biochem.*, **23**, 341 (1949).

(4) M. L. Anson and A. E. Mirsky, *J. Gen. Physiol.*, **17**, 151 (1933).

(5) H. T. Huang and C. Niemann, THIS JOURNAL, **73**, 1541 (1951).

(6) D. W. Thomas, R. V. MacAllister and C. Niemann, *ibid.*, **73**, 1548 (1951).

(7) H. Lineweaver and D. Burk, *ibid.*, **56**, 608 (1934).