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]

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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).

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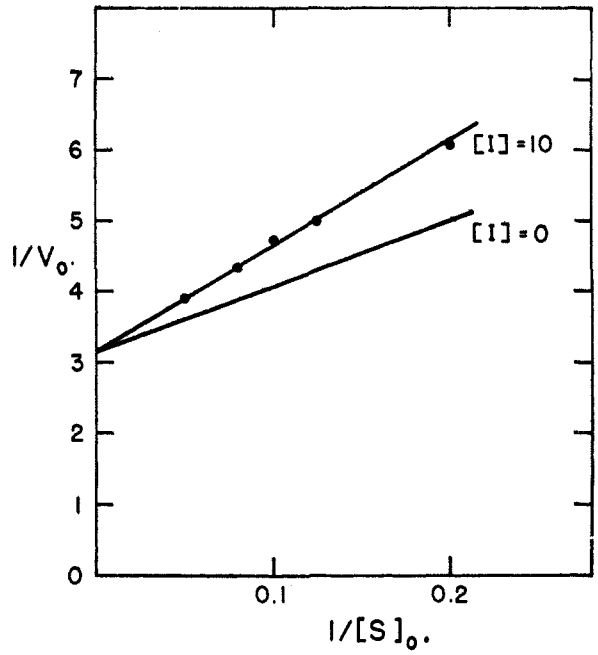

Fig. 1.—Inhibition of the α-chymotrypsin-catalyzed hydrolysis of nicotinyl-L-tryptophanamide by chloramphenicol: $[S]_0$ in moles × 10^{-3} per liter, [I] in moles × 10^{-3} per liter, v_0 (initial velocities) in moles × 10^{-3} per liter per min., [E] = 0.208 mg. protein-nitrogen per ml.

under the same conditions, and at an initial substrate concentration of 20 × 10^{-3} M, gave a mean value for K_I of 13.0 × 10^{-3} M, with K_S in this instance equal to 30.5 × 10^{-3} M.[6] With K_I for the system α-chymotrypsin–chloramphenicol of the order of 10 × 10^{-3} M it is not surprising that Smith and Worrel[3] could not detect any inhibitory action at chloramphenicol concentrations of 0.69 × 10^{-3} M with the methods used in their study.

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Preparation of Dibutyl Allylphosphonate

BY GENNADY M. KOSOLAPOFF

Some time ago Pudovik[1] showed that dialkyl sodiophosphites may add to the double bond of an alkenephosphonate, thus yielding diphosphonates. Such a reaction was observed by him in a number of instances involving the reaction of dialkyl sodiophosphites with aliphatic halides of allyl type. More recently, Rueggeberg and co-workers[2] isolated a tetrabutyl propanediphosphonate from the reaction of allyl bromide and dibutyl sodiophosphite. Since the latter workers failed to state whether or not any of the monosubstitution reaction product was obtained, it was of some interest to look into the possibility of isolation of the allylphosphonate from this reaction. This was done readily, although in disappointing yields, by using the sodiophosphite prepared from the free metal and under conditions in which the free phosphite was present in a slight excess.

Experimental. 1.—Dibutyl phosphite (98.0 g.) was allowed to react with 11.5 g. of sodium in 500 ml. of hexane and the resulting solution was treated over 30 minutes with 39 g. of allyl chloride. The mixture was gently refluxed for 30 minutes and was treated with 100 ml. of ice-water, with external cooling. The upper layer was combined with a hexane extract of the lower part of the mixture and the combined hexane solution was washed with three 50-ml. portions of ice-water. Distillation gave 16.5 g. of the crude product, b.p. 151° at 5 mm. The reaction was repeated, with a six-hour refluxing period of the mixture and with an increase of the amount of the original wash water to 500 ml.; the yield of the crude product was 15 g. In both instances the bulk of the distillation residues was the high-boiling diphosphonate reported earlier.[2] Fractionation of the combined products gave 17 g. of dibutyl allylphosphonate, as a colorless liquid, b.p. 110° at 0.4 mm., $n^{25}D$ 1.4336, d^{25}_{25} 0.9548. Found: P, 13.3; MR, 63.7. Calcd. for $(BuO)_2P(O)CH_2CH{:}CH_2$: P, 13.24; MR, 63.4.

2.—The reaction with allyl bromide gave similar results. Addition of 61 g. of allyl bromide to a refluxing solution of 99 g. of dibutyl phosphite and 11.5 g. of sodium in 400 ml. of xylene in the course of three hours, followed by cooling and washing as above, gave 39 g. of dibutyl allylphosphonate, b.p. 111 at 0.5 mm. Again, the bulk of the distillation residue consisted of the above-mentioned diphosphonate.

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Isolation and Identification of Pyrrole and 2-Methylpyrrole from Shale Oil[1]

BY A. G. JANSSEN, E. R. SCHIERZ, R. VAN METER AND JOHN S. BALL

Shale-oil products before treatment generally show excessive color and gum formation. This has been attributed to the presence of pyrrole-type compounds. To investigate this problem further, the particular compounds present should be known. In this research pyrrole and 2-methylpyrrole were isolated and identified from an oil prepared from Colorado oil shale.

Pyrroles, as a class, have been identified in shale oil[2] by the pine-splint test and the isatin test. However, there is no record of previous identifications of individual compounds. This is probably due to the reactivity of the compounds as evidenced in this research by low recoveries from each stage of the separation.

The shale oil studied was produced from Colorado shale of the Green River formation by retorting in an N-T-U retort.[3] Material boiling up to 195° was separated by distillation, and the tar acids were removed by treatment with dilute sodium hydroxide. Pyrroles were then separated as potassium pyrroles by refluxing the naphtha over solid potassium hydroxide. The potassium compounds were washed with pentane and hydrolyzed. The recovered oil was distilled, and derivatives were made from some of the fractions. The phthalic anhydride and tetraiodo derivatives of pyrrole were thus isolated and compared with authentic sam-

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