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A NEW PROCEDURE FOR THE SYNTHESIS OF L-HISTIDINOL AND ITS APPLICATION IN PREPARING ^{14}C L-HISTIDINE

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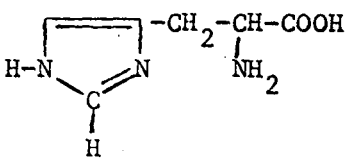
DISCLAIMER

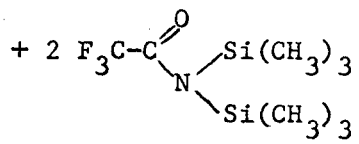
A new procedure for the synthesis of L-histidinol and its application in preparing [^{14}C]L-histidine

L-histidinol is an intermediate in the histidine biosynthetic pathway and, as such, is of use to investigators studying this pathway. Although unlabeled histidinol is now commercially available from several sources, labeled histidinol is not. We have developed a new method for the chemical preparation of this compound, which we believe to be much less involved and to give somewhat better yields than the previously published procedure[1]. Using this method we prepared [^{14}C]L-histidinol from tracer amounts of [^{14}C]L-histidine.

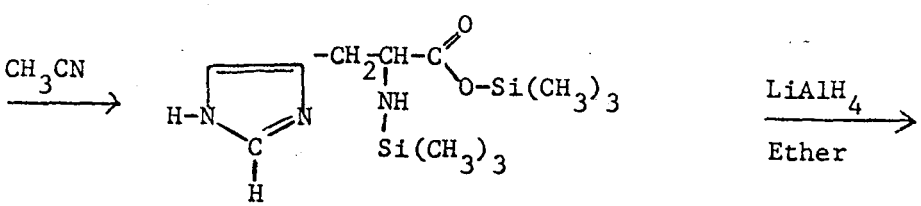
Principle:

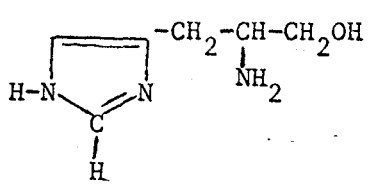


[^{14}C]L-histidine



BSTFA





[^{14}C]L-histidinol

Although both our method and the previously published one[1] consist essentially in the reduction of the carboxyl group of L-histidine with lithium aluminum hydride, we have eliminated several tedious steps of derivatization of the amino and carboxyl groups and subsequent removal of the side chains by using the silylation reagent bis(trimethylsilyl)-trifluoroacetamide (BSTFA). This reagent reacts with both functional groups[2] and both the reagent and the derivatives hydrolyze by mere contact with water.

Procedure

Approximately 50 µcuries/0.03 mg [^{14}C]L-histidine (New England Nuclear, not checked for purity) is used as a starting material. The histidine solution is transferred to a conical centrifuge tube fitted with a ground joint that can be stoppered or attached to a small condenser. Thus, all reactions can be carried out in the same tube. The histidine is brought to dryness under a stream of nitrogen, then heated for 1 h in a 70°C oven. Ten µl (a 100-fold excess) of BSTFA ("Regisil", Regis Chemical Co.) are transferred rapidly, using a microsyringe, from the original vial to the reaction tube. Twenty µl acetonitrile, previously dried by passing it in succession over $CaSO_4$, $MgSO_4$ and molecular sieve, are also added to the reaction mixture. The tube is stoppered and incubated in an oil bath at 150°C for 2 - 2-1/2 h. The oil bath should be made out of a wide-mouthed container, so that only the tip of the centrifuge tube is immersed in the bath and the air just above the oil surface is fairly cool. The reaction tube will then serve as a condenser and the reaction mixture will reflux within the tube. This silylation was adapted from the procedure of Gehrke et al.[3].

$LiAlH_4$ solution is prepared by stirring the solid material with anhydrous ether, in a nitrogen box, for about 1 h. A rough quantitation of the solution is then made by reacting an aliquot of the supernatant slurry with a standard HCl solution, which is then backtitrated with NaOH.

The above reaction mixture is cooled on ice and an excess of the $LiAlH_4$ solution (taking into account the possible reaction of the acetonitrile with $LiAlH_4$[4]), for example, 0.4 - 0.5 mmoles for the quantities given above, is added carefully to the tube. When the strong bubbling has ceased, the tube is attached to a small condenser, on the top of which passes a stream of nitrogen, and the mixture is gently refluxed for about 1 h. The reaction mixture is then cooled on ice and carefully hydrolyzed with an excess of water, until no more bubbling is observed. The ether is blown off and the precipitated hydroxides removed by centrifugation from the aqueous supernatant containing most of the product. The precipitates can be washed one or two more times with water, until all the counts are in the supernatant.

At this stage, the supernatant still contains a quantity of dissolved aluminum hydroxide which would interfere with the paper chromatographic procedure used for the purification of the product. The following "desalting" procedure is therefore applied: The volume of the solution is reduced by evaporation to about 0.1 ml. Acetonitrile is added, drop by drop, to about 1 - 2 ml, or until no more salting out is observed. The "salt" is removed by centrifugation. About 20% of the counts remain in the precipitate at this point; if desired, these can be recovered by redissolving the precipitate in water and repeating the above procedure.

The acetonitrile-water supernatant containing the product can be spotted directly on paper for chromatography ("Ederol" No. 202 paper, J. C. Binzer Filtrierpapier-Fabrik, Hatzfeld/Eder, Germany).

The following two-dimensional chromatographic system is used for purification of the product:[5] 1) iso-propanol-water-ammonia (200:20:10, by vol.); 2) n-butanol-glacial acetic acid-water (120:30:50, by vol.). The chromatogram is autoradiographed and the [^{14}C] histidinol spot, representing about 70% of the input counts is eluted with water or dilute HCl. The location of the labeled histidinol can be confirmed by co-chromatography with commercial carrier L-histidinol. We have measured an overall yield of histidinol of 55-60%. However, this figure is not too accurate (and probably low) because our counting was done in a scintillation system and histidinol appears to be highly insoluble in the various organic solvents.

The identity of the product was confirmed by two-dimensional chromatography on thin-layer plates, in the following system:[5] 1) n-butanol-acetone-diethylamine-water (70:70:14:35, by vol.); 2) isopropanol-formic acid-water (40:2:10, by vol.). It was also confirmed biologically, in the following way: Salmonella typhimurium G-46, a histidine-requiring mutant, which can metabolize histidinol, was grown on carrier L-histidinol (Cyclo Chemicals) with some of our labeled product added. Aliquots were removed from the culture at various time intervals and precipitated with trichloroacetic acid. The incorporation of counts was linear with cell mass and agreed very well with the theoretical value calculated from the known specific activity of the medium, the amount of protein present and the proportion of the protein represented by histidine. The trichloro-acetic acid precipitate was also heated, washed, hydrolyzed, and

chromatographed, as described elsewhere,[6] and the radioactivity was shown to appear with the histidine spot.

Because of the small amount of material present, we have not measured the specific rotation of the product. However, Karer, Portmann and Suter[7] have shown that reduction of optically active esters in which the α-carbon atom is asymmetric, as in amino acids, occurs without racemization. The fact that our compound is biologically active also argues in this direction.

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