Stereospecific Synthesis of Isotopically Labeled Serine at Carbon 3 and Stereochemical Analysis of D-Serine Dehydrase Reaction

Sir:

We report here a straightforward synthetic scheme producing enantiomeric pairs of [3-^{2}H]- or [3-^{3}H]serine on a gram scale and the determination of the absolute configurations of the chiral preparations. We also illustrate one of its utilities by probing the stereochemical course of the elimination reaction catalyzed by the pyridoxal phosphate-dependent enzyme D-serine dehydrase from *E. coli*. While synthesis of either [3-^{3}H]serine[1a] or [3-^{2}H]serine[1b,c] has recently been reported in preliminary form, the tritiated amino acid was obtained in a low yield on a microscale after many enzyme-catalyzed steps and is not amenable to scale up beyond a few micromoles. The synthesis of the [3-^{2}H]-compound reportedly involves hydrogenation of an olefinic azlactone of presumed geometry, leading subsequently to amino acid of which the configuration at carbon 3 has not been unambiguously established.

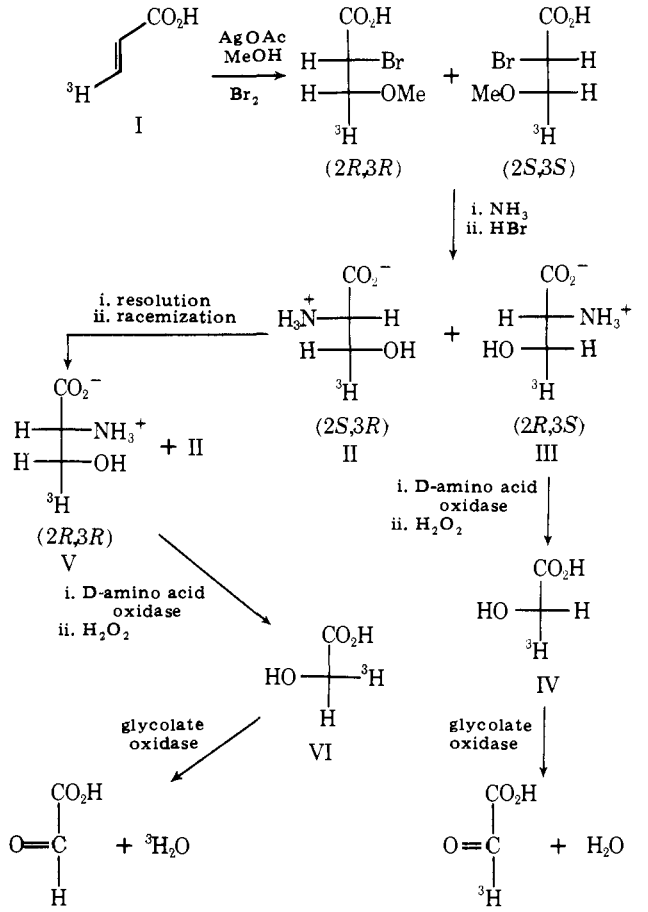
The synthesis outlined in Scheme I was initiated by decarboxylation of 1 g of monopotassium acetylene dicarboxylate in 2.5 ml of ^3H_2O (200 mCi).[2] After removal of excess ^3H_2O, the residue, without purification, was reduced with Cr^{II} sulfate.[3] Carrier acrylic acid (1 g) was added and the (*E*)-[3-^{3}H]acrylic acid[3] isolated by continuous extraction into ether, followed by distillation in quantitative yield (59 000 cpm/μmol). Conversion to the enantiomeric pair of (2*R*,3*S*)- and (2*S*,3*R*)-[3-^{3}H]serine was performed by a modified literature procedure.[4] $Ag(OAc)_2$ (2.3 g) was suspended in a solution of 1 g of I in 20 ml of absolute methanol and cooled to −10 °C. With vigorous stirring 2.2 g of Br_2 in 5 ml of methanol was added dropwise over the course of 1 h. Subsequent procedures were as reported[4] and the intermediates were not isolated. A yield of 0.8 g (55%) of an enantiomeric pair of [3-^{3}H]serines was afforded after purification on a Dowex 50 H^+ column and recrystallization from aqueous ethanol.

The absolute configurations at C2 and C3 were established as (2*S*,3*R*) (II) and (2*R*,3*S*) (III) by enzymatic oxidations of known stereospecificity. Hog kidney D-amino acid oxidase (18 U/mg)[5] oxidized only those serine molecules of 2*R* configuration and, after removal of unreacted [^{3}H]serine by Dowex 50 chromatography and decarboxylation of the 3-hydroxy[3-^{2}H]pyruvate by H_2O_2, the [2-^{3}H]-glycolate was treated exhaustively with spinach glycolate oxidase (0.25 U/mg),[6] known to remove only the pro-*R* hydrogen of glycolate.[7] Only 20% of the tritium was released as ^3H_2O, establishing that 80% of the glycolate molecules contained tritium in the pro-*S* position (IV), showing in turn that the (2*R*,3*S*)-[3-^{3}H]serine molecules were 80% enantiotopically pure at carbon 3.

Proof that the expected enantiomer (2*S*,3*R*)serine (II) was present followed by sequence in Scheme I also. II was resolved from III by specific hydrolysis of the *N*-acetyl derivative with hog kidney acylase[8] followed by racemization at carbon 2[9] to a mixture of II and V. The D-amino acid oxidase-H_2O_2 protocol selectively oxidized V to a [2-^{3}H]glycolate which was 80% VI since 80% of the tritium was removed as ^3H_2O after glycolate oxidase treatment. Thus the (2*S*,3*R*)-[3-^{3}H]serine enantiomer present is also 80% enantiotopically pure at carbon 3. The lack of absolute chiral purity may arise either during addition of the bromo and methoxy groups or the amination step[10] and is under investigation.

With the same methods an enantiomeric mixture of (2*R*,3*S*)- and (2*S*,3*R*)-[3-^{2}H]serine was prepared from (*E*)-[3-^{2}H]acrylic acid.[3] Examination of its deuterium-

Scheme I



decoupled NMR spectrum in alkaline ^2H_2O allows us to assign unambiguously the upfield doublet[1c] to the C3 pro-*R* proton of L-serine and the C3 pro-*S* proton of D-serine.

The chiral serines can be used to probe the stereospecificity of a variety of enzymatic reactions occurring at the C3 of serine: for instance Floss and his colleagues have shown tryptophan is formed with retention of configuration.[1a]

One of the intriguing unresolved problems in pyridoxal phosphate enzymology is whether during β-eliminations reprotonation at the substrate β-carbon is enzyme-catalyzed.[11] Recently, Yang et al.[12] found that D-threonine was converted predominantly to (3*S*)-2-keto-[3-^{2}H]butyrate by D-serine dehydrase in ^2H_2O, a retention of configuration. As an initial test for our chiral tritiated serines, we have now used that *E. coli* D-serine dehydrase (>100 U/mg) in ^2H_2O and show here the first proof that a pyridoxal phosphate enzyme catalyzes the formation of a chiral methyl group during a β-elimination reaction.

Thus, separate incubations of III or V as dehydrase substrates produced chiral pyruvates VII and VIII, respectively, analyzed as indicated in Scheme II by eventual conversion to [3-^{3}H]malate samples. To improve the accuracy of the results authentic [2-^{14}C]malate was added to the tritiated samples which were then purified as previously reported[13] before exhaustive treatment with fumarase.[14] Both the ^3H_2O and the $^3H/^{14}C$ ratios of malate (before and after fumarase action) were measured, yielding the results of Table I.

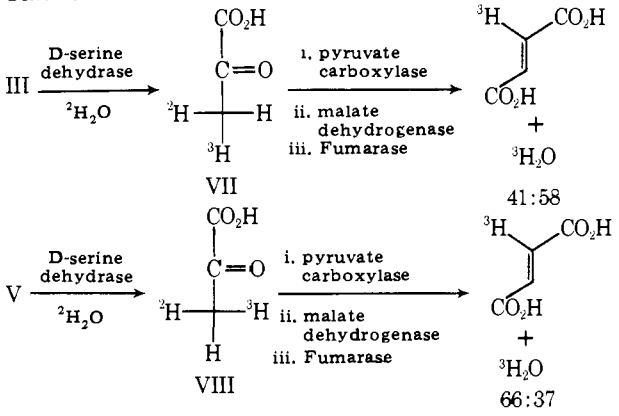
Given (2*R*)-serines of 80% enantiotopic purity at carbon 3 and the *intra*molecular deuterium isotope effect of 3.1 for chick liver pyruvate carboxylase,[15a,b] the maximal chiral enrichment at C3 of malate, for 100% reprotonation of bound aminoacrylate at the active site of D-serine dehydrase before release into solution, would produce a 64:36 split after fumarase action.[16] The data of Table I approach

Table I. Fumarase-Treated [2-^{14}C,3-^{3}H]Malate[a]

Derived from	% tritium released as $^{3}H_2O$	% tritium retained[b]
III	58	41
V	37	66
V in $^{1}H_2O$[c]	48	53

[a] See ref 13. About 2 μmol of [3-^{3}H]malate were prepared in each incubation. A typical set of data: e.g., with the [2-^{14}C,3-^{3}H]-malate from V (^{3}H, 15 000 cpm; ^{3}H/^{14}C ratio, 1.57) 5560 cpm of $^{3}H_2O$ was released and the ^{3}H/^{14}C ratio dropped to 1.02. [b] Calculated from the ^{3}H/^{14}C ratios of malate samples before and after fumarase treatment. In addition to the paper chromatograph system used in ref 13, malate samples were also purified on Whatman No. 3 paper, developed in ethanol/ammonia/water (8:2:1), R_f = 0.24. [c] Control experiment, in which a 50:50 partition should be expected.

Scheme II



such chiral enrichment and most importantly compounds III and V yield the expected reciprocal patterns.

These results show that D-serine dehydrase reprotonates the *bound* aminoacrylate, and with retention of configuration with respect to the departed hydroxyl group. They also validate the use of these chirally labeled serine preparations as probes of stereochemistry. The apparent lack of absolute stereospecificity may be due to the occasional release of the aminoacrylate, which tautomerizes free in solution, and thus incorporates a proton achirally.

After the submission of this manuscript, a complementary report has appeared, using chirally labeled [3-^{3}H]serines to determine the stereochemical path for elimination reactions catalyzed by tryptophanase and tryptophan synthetase.[19]

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References and Notes

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(15) (a) A tentative value of 3.3 was quoted by Rose in ref 14. (b) Y. F. Cheung and C. Walsh, unpublished results.
(16) Based on the assumption that the pyruvate molecules are only 95% deuterated. In our actual incubations, the solution contained about 3% $^{1}H_2O$.
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Book Reviews

Concepts of Inorganic Photochemistry. By ARTHUR W. ADAMSON (University of Southern California) and PAUL D. FLEISCHAUER (The Aerospace Corp.). John Wiley & Sons, Inc., New York, N.Y. 1975. xiii + 430 pp. Price $22.50.

In the extremely rapidly moving area of inorganic photochemistry, this is an important and useful book. It is stimulating and readable and should find its way onto the shelves of most inorganic photochemists and spectroscopists as well as newcomers to the area. It will not replace "Photochemistry of Coordination Compounds" as a reference book, but it seems to fill in most of the important references through 1974 and the newly emerging concepts; its format is also probably better for teaching a course in inorganic photochemistry for a reasonably sophisticated audience.

It has chapters on energy levels and spectra, photokinetics, charge-transfer photochemistry, substitutional photochemistry of first-row transition elements, and photochemistry of the heavier elements, of carbonyl complexes, of 1,3-diketonates, of simple inorganic ions, and of the solid state. It concludes with chapters on photochromism and chemiluminescence. Each chapter is written by a different author with the last two by the editors. Unlike the usual hodge-podge of many multiauthored books, an overall plan is carefully followed, and overlap and inconsistent notation are minimal. Although the writing and level of presentation are not always consistent, the problems are acceptable. For alternate viewpoints and additional references, the reviews by Wrighton on carbonyls (*Chem. Rev.*, **74**, 401 (1974)) and on sensitization by Balzani et al. (*Coord. Chem. Rev.*, **15**, 321 (1975)) are recommended.

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