A Convenient Synthesis of 2S,3S-[3-^{2}H]-Serine and 2S,3R-[2,3-$^2H_2$]-Serine

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ABSTRACT

Both 2S,3R-[2,3-$^2H_2$]-serine 5 and 2S,3S-[3-^{2}H]-serine 6 have been prepared from (E)-methyl-[2,3-$^2H_2$]-acrylate and (Z)-ethyl-[3-^{2}H]-acrylate, respectively. The acrylate esters were converted to a mixture of isomeric bromohydrins by treatment with N-bromoacetamide. The ratio of 2-bromo-3-hydroxy species to the 2-hydroxy-3-bromo isomer was approximately 3:1. Conversion to the corresponding azido alcohols by treatment with NaN_3 followed by catalytic reduction over Pd gave the alkyl esters of serine and isoserine. Purification and hydrolysis yielded serine in typically 20-25% yield from methyl or ethyl acrylate. Resolution was accomplished enzymatically by hog kidney acylase I treatment of the N-acetyl derivative. Absolute configuration was determined by ^{1}H NMR and the ratio of proton intensity in the diastereotopic positions at C-3 were measured to be H_S/H_R = 11 for 5 and H_R/H_S = 4.4 for 6.

Key words: 3R and 3S [3-^{2}H]-serines. Amino acids, deuterium labelling, Enantiomeric synthesis. Chiral synthesis.

INTRODUCTION

Isotopically labelled amino acids of high stereochemical purity are extremely useful in the study of biological systems, especially in the elucidation of enzymatic mechanisms. We report here a scheme for synthesizing in high chemical and enantiomeric yield both 3R-[2,3-$^2H_2$]-serine 5 and 3S-[3-^{2}H]-serine 6, an amino acid important in one-carbon metabolism (1). Both [3-^{2}H](2a,b) and [3-^{3}H](3)-serine have been reported previously, but the procedures leading to these compounds all suffer from limitations. The tritiated material was

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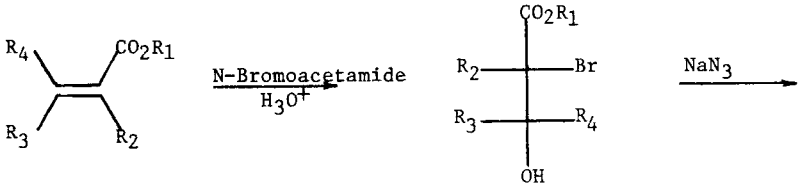
prepared enzymatically from either D-[1-^{3}H]-glucose or D-[1-^{3}H]-mannose through 3-phosphoglycerate, an intermediate in the glycolytic pathway and although the procedure resulted in a product of high stereochemical purity, it is not amenable to a large scale up. The synthesis of the deuterated serine made use of 1S-[1-^{2}H]-2-phenyl-ethanol as starting material and yielded serine in 7 steps with no apparent loss of integrity at the initial chiral center. However, the synthesis of the starting alcohol involved microbiological conversion of tyramine to 1S-[1-^{2}H]-tyrasol, followed by reduction of the phenolic hydroxyl through a tetrazole intermediate (5), thus complicating the synthesis considerably. More recently Cheung and Walsh (6) reported the chemical synthesis of only the 3R isomer of both [3-^{3}H] and [3-^{2}H] serine. This procedure resulted in high overall chemical yield, but a somewhat poorer stereochemical purity (60% enantiomeric excess at C-3). Furthermore, the second step in the synthetic scheme proceded in very low yield, and although this can be overcome for tritiated material by incorporating a very high specific activity, it severely limits the facility in producing deuterated serine.

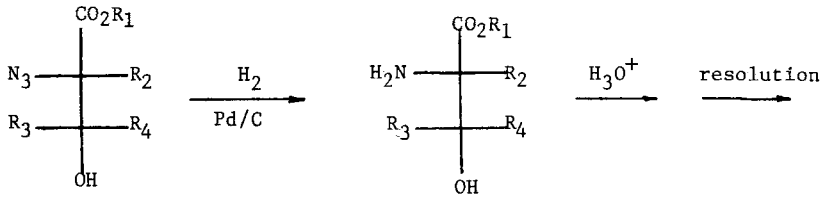
RESULTS AND DISCUSSION

We present a synthesis of [3-^{2}H]-serine starting with deuterium labeled acrylate esters prepared by the method of Hill and Newkome (7) (Scheme I). This procedure generates [^{2}H]-acrylate esters regioselectively by first condensing the corresponding propiolate ester with anthracene in a Diels Alder fashion, and then hydrogenating the adduct catalytically over Pd. Pyrolysis at 300° yields the free acrylate from a retro-Diels Alder reaction. The net result is a cis hydrogenation of an alkyne to an alkene, without the problems of trans hydrogenation or over reduction to the alkane. Using this method, both

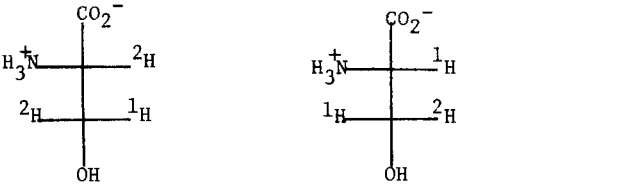
Scheme I[a]



1a,b 2a,b



3a,b 4a,b



5 2S,3R-[2,3-$^2H_2$]-serine 6 2S,3S-[3-^{2}H]-serine

a $R_1 = CH_3$, $R_2 = R_3 = {}^2H$, $R_4 = {}^1H$

b $R_1 = CH_2CH_3$, $R_2 = R_3 = {}^1H$, $R_4 = {}^2H$

[a] For species 2,3 and 4, only one enantiomer of a racemic mixture is shown. The 2-hydroxy regio isomers of 2,3 and 4 are not shown.

(E)-methyl-[2,3-$^2H_2$]-acrylate 1a and (Z)-ethyl-[3-^2H]-acrylate 1b were prepared cleanly from methyl propiolate and ethyl-[3-^2H]-propiolate, respectively. The latter compound was prepared from ethyl propiolate and NaOD in D_2O/EtOD by exchange of the acidic acetylenic proton (8). Treatment of the acrylate esters with N-bromoacetamide (NBA) in dilute H_2SO_4 (9) generated two isomeric bromohydrins in typically 60% yield after vacuum distillation. GC/MS analysis (see Experimental for conditions) confirmed the identity of the two species as the 2-bromo-3-hydroxy and the 3-bromo-2-hydroxy isomers in a ratio of 3:1 respectively. The peak assigned as the 2-bromo-3-hydroxy isomer showed a base peak in the mass spectrum (CI) at m/e 167,169 indicating loss of H_2O. The spectrum of the other isomer showed this peak at much lower intensity. Protonation of OH during chemical ionization followed by expulsion of H_2O would be expected to be favored on the isomer that could place the positive charge β to the carbonyl in the resulting ion. Electron impact mass spectrometry (70 eV) gave no useful information. Quantitative conversion of the mixture of bromohydrins to the corresponding azido alcohols was accomplished by treatment with NaN_3 in H_2O/EtOH at reflux for 1 hr (10). Reduction of the azide moiety to an amine was effected by hydrogenation over 10% Pd/C in ethanol. Flash column chromatography using 230-400 mesh silica gel and 8:2 (v/v) CH_2Cl_2/MeOH as eluent separated the esters of serine and isoserine quantitatively as indicated by TLC and GC. Aqueous acid hydrolysis followed by Dowex 50 chromatography (H^+ form) and recrystallization from ethanol gave pure serine in the zwitterionic form. Resolution of the D,L mixture was accomplished by hog acylase I treatment of the N-acetyl derivative (11).

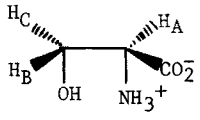
A stereochemical analysis of L-serine in aqueous solution using ^1H nmr has already been reported (12). Using these data it is possible to unambiguously

determine the absolute stereochemistry at C-3 for a stereospecifically labeled [3-^{2}H]-serine. The data from the 360 MH_z ^{1}H spectra of both 5 and 6 are tabulated in Table I. Looking first at the data for 2S,3R-[2,3-$^2H_2$]-serine, it is readily apparent that incorporation of ^{2}H into the α position (H_A) and into the pro-R position at C-3 (H_C) is quite high. The 8% ^{1}H residue at the α position is presumably due to incomplete removal of H_2 from the catalyst surface during the catalytic deuteration of the Diels-Alder adduct in the Hill procedure. The ratio, therefore, of hydrogen at C-3 in the pro-S position to that in the pro-R position is equal to 11 and is a direct measure of the stereochemical integrity at that position.

The spectrum of 2S,3S-[3-^{2}H]-serine is more complex due to the greater number of protons present. Ideally, the spectrum should consist of two doublets with equal coupling constants corresponding to H_A and H_C. This will be complicated by resonances from non-deuterated material present in an amount dependent on the initial deuterium incorporation of the starting ethyl propiolate, as well as by shielding effects on the proton chemical shift due to a neighboring deuterium nucleus. As can be seen in Table I, at 360.13 MH_z the discrete resonances of H_A, H_B^*, H_C and H_C^* can be resolved (see Table for explanation of symbols). The shielding effect on δH_C when H_B = ^{2}H is 8.6 H_z (0.024 ppm) and the total integration of H_β relative to H_α is 1.13, indicating that approximately 13% of the total serine pool is non-deuterated. Although H_B is not totally resolved from H_B^*, the apparent ratio of proton intensity at C-3 in the pro-R position to that in the pro-S position is 4.4. This value is considerably lower than that determined for the 3R isomer, and presumably must reflect to a large extent poor incorporation of deuterium into the starting ethyl acrylate. NMR integration of ethyl-[3-^{2}H]-propiolate showed 95% deuterium incorporation

Table I

360.13 MHz ^1H NMR Data of [3-^2H]-serines[a]



Serine	Proton	Chemical Shift in ppm[6] (multiplicity)	J (H_z)[c]	Integration[d]
	H_A	3.314 (d)	J_{AB} = 5.8	0.083
5 (3R)	H_B	3.649 (s)		1.0
	H_C	3.706 (s)		0.094
	H_A	3.314 (d)	J_{AB} = 5.6	1.0
6 (3S)	H_B^* + H_B	3.615 (dd)	J_{BC} = 11.4, J_{AB} = 5.6	0.21
	H_C	3.712 (d)	J_{AC} = 4.1	0.92
	H_C^*	3.735 (dd)	J_{BC} = 11.4, J_{AC} = 4.1	

[a] Samples were 38 mM in L-serine, pD ~ 12.5.
[b] Relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).
[c] Discrete ^1H - ^2H coupling was not observed due to its magnitude (approximately 15% of the corresponding ^1H - ^1H coupling constant) and was assumed to contribute only to linewidth.
[d] Spectrometer line integration.
[e] Asterisk (*) denotes proton on non-deuterated serine.

which dropped to 90% in ethyl-[3-^{2}H]-acrylate. This is probably within the error of integration, but may reflect a partial washing out of deuterium during the Pd catalyzed hydrogenation of the Diels-Alder adduct, as suggested by Aberhardt (13) in the synthesis of n-butyl acrylate. If one assumes that the only species present are 3S-[3-^{2}H], 3R-[3-^{2}H] and non-deuterated serine, then the value of 3R/3S, corrected for non-deuterated material, is equal to 10. This closely approximates the value for the 3R isomer of 11, and verifies the stereochemical integrity of the synthetic procedure.

We have reported a simple synthesis of stereospecifically labeled [3-^{2}H]-serine in approximately 20% overall yield from labeled methyl or ethyl acrylate. The stereochemical integrity of the C-3 center is quite high for the 3R isomer and somewhat lower for the 3S, reflecting the poorer initial deuterium incorporation in the latter compound.

EXPERIMENTAL SECTION

Infrared spectra were recorded on a Perkin-Elmer Model 735 spectrometer, while routine 60 MHz NMR spectra were taken on a Varian EM-360 using TMS as an internal standard. High resolution NMR spectra were run on a Brüker WM-360 operating at 360.13 MHz for ^{1}H, using sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal reference.

Mass spectral analyses were performed on a Kratos MS9 using electron impact (EI) ionization.GC/MS analyses were performed on a Finnegan 9500 gas chromatograph coupled to a model 3200 mass spectrometer employing chemical ionization (CI). Separations were performed at 150° on a column packed with 10% Silar 10-C on Chromosorb W. Unless otherwise stated, all routine GC analyses were determined on a Varian Model 3700 gas chromatograph employing flame ionization

detection and a 3% OV-225 on Chromosorb 100 column at 150°. N-Bromoacetamide was purchased from Aldrich and recrystallized from $CHCl_3$ and hexane prior to use. Silica gel (230-400 mesh) was obtained from E. Merck, Darmstadt, W. Germany. Anthracene was purchased from Aldrich and recrystalized from ethyl acetate prior to use.

(E)-methyl-[2,3-$^2H_2$]-acrylate 1a. A mixture of 14.9 g anthracene (83.7 mmol) and 7.6 g methylpropiolate (90.5 mmole) in 40 ml m-xylene was refluxed under N_2 for 7 days. The Diels-Alder adduct was isolated as reported previously (7) in 50% yield. Catalytic hydrogenation under D_2 using carefully prepared catalyst (14) (10% Pd on C) in anhydrous ethyl acetate yielded the reduced adduct quantitatively. Mass spectral analysis indicated the following: m/e 265, 4.9%, 266(M^+), 100%, 267, 19.2%. Pyrolysis of this material, followed by simple distillation, gave 2.6 g of 1a (75%). NMR ($CDCl_3$, internal TMS) δ 3.67 (s,3), δ 6.32 (t,1,J = 2.6 H_z, R_4).

(Z)-Ethyl-[3-^{2}H]-acrylate (1b). The Diels-Alder adduct of ethyl-[3-^{2}H]-propiolate (8) and anthracene was prepared as reported previously (15). Catalytic hydrogenation under H_2 gave the reduced adduct, which upon pyrolysis generated 1b (78%) NMR ($CDCl_3$, internal TMS) δ 1.29 (t,3,J = 7 H_z), δ 4.19 (q,2,J = 7 H_z), δ 5.70 (d,1,J = 11 H_z, R_3), δ 5.97 (dt,1,J = 3, 11 H_z, R_2). Total vinylic region = 2.1 H or 90% incorporation of one ^2H.

(±)-Methyl-2-bromo-3-hydroxy-[2,3-$^2H_2$]-propionate 2a. To a solution of 6.38 g N-bromoacetamide (46.2 mmole) in 50 ml of 0.4 M H_2SO_4 was added dropwise 2.6 g 1a (30.2 mmol). This was stirred vigorously at 5° for 1.5 hours and then extracted 3X with CH_2Cl_2 (150 ml total). The organic layer was extracted once with 5% $NaHSO_3$, dried over $MgSO_4$ and reduced by rotary evaporation to a

clear oil. Purification by vacuum distillation (57°@ 1 mm Hg) gave 3.07 g (16.3 mmol, 56%) of product. Gas chromatographic analysis indicated only two components with retention times equal to 1.3 and 1.8 min in a ratio of 1:3. GC/MS analysis on the non-deuterated material confirmed the assignment as the 3-bromo-2-hydroxy and 2-bromo-3-hydroxy isomers, respectively. IR (mixture, neat), 3448,1739 cm^{-1}.

(±)-Ethyl-2-bromo-3-hydroxy-[3-^{2}H]-propionate 2b. The above procedure was repeated with 3.06 g (31.2 mmol) of 1b and 5.9 g (42.7 mmol) NBA. Yield after vacuum distillation (59-61° @ 0.9 mm Hg) was 3.96 g (20.1 mmol, 64%). GC analysis indicated only two components, t_R = 1.6 and 2.2 min under the same conditions as above. Again, the areas were in a ratio of 1:3, and were assigned as above.

(±)-Methyl-2-azido-3-hydroxy-[2,3-$^2H_2$]-propionate 3a. NaN_3 (1.80 g, 27,7 mmol) was dissolved in 7 ml H_2O. To this was slowly added 11 ml abs ethanol. Addition of 2a (3.06 g) was followed by reflux for 1 hour. Workup was as reported previously (10). GC analysis indicated major peaks at 1.5 and 2.7 min, with a small amount of unreacted 3-bromo-2-hydroxy starting material remaining. The amber product was not purified further. IR (neat), 3400, 2120, 1739 cm^{-1}.

(±)-Methyl-2-amino-3-hydroxy-[2,3-$^2H_2$]-propionate 4a. The azido ester 3a was dissolved in 25 ml absolute ethanol and reduced catalytically over 200 mg of 5% Pd/C. After 2 hours at room temperature, an aliquot was removed, filtered of catalyst and analyzed by GC. Starting material had been replaced quantitatively by product at t_R = 0.95 and 1.4 min. TLC (Silica, 8:2 CH_2Cl_2/MeOH) indicated two components: R_{f1} = 0.39 (orange to ninhydrin) and R_{f2} = 0.77 (yellow to ninhydrin). These were shown to correspond to the methyl esters of isoserine

and serine, respectively, by comparison to authentic standards.

(±)-Ethyl-2-azido-3-hydroxy-[3-^{2}H]-propionate 3b and (±)-Ethyl-2-amino-3-hydroxy-[3-^{2}H]-propionate 4b. Essentially the same procedure was followed as described above for the corresponding methyl esters.

Column purification. Medium pressure silica gel column chromatography was employed to separate the esters of serine and isoserine (4 x 20 cm, 230-400 mesh silica gel, 8:2 CH_2Cl_2/MeOH). A flow rate of approximately 60-100 ml min^{-1} was maintained by positive N_2 pressure. Fractions containing the methyl or ethyl ester of serine (yellow to ninhydrin) were pooled and shown to be pure of 2-hydroxy isomer by both GC and TLC. Product generally eluted with a retention volume of between 500 and 1000 ml. Rotary evaporation of the pooled material gave a pale yellow oil.

2S,3R-[2,3-$^2H_2$]-serine 5 and 2S,3S-[3-^{2}H]-serine 6. To effect hydrolysis, 4a and 4b were each dissolved in 60 ml 1N HCl and refluxed under N_2 for 5 hours. TLC (silica, 4:4:1 $CHCl_3/CH_3OH/NH_4OH$) indicated no visible ester remaining. Conversion to the zwitterion was accomplished by loading the condensed sample (evaporated to dryness and redissolved in 5 ml H_2O) onto a Dowex 50 (H^+) column (20 x 1 cm. 200-400 mesh) followed by extensive washing with H_2O and elution with 1.5 N NH_4OH. Recrystallization from H_2O/ethanol gave pure D,L-serine in typically 20-25% overall yield from methyl or ethyl acrylate. Resolution via hog acylase I treatment of the N-acetyl derivative proceeded in 85-95% yield (11). Final yields of 5 and 6 were 230 mg and 354 mg respectively.

Notes and References

1. R.L. Blakely, "The Biochemistry of Folic Acid and Related Pteridines", North Holland Publishing Company, Amsterdam, 1969. Chpt. 8.

2. a) C. Fuganti et al, Chim. Ind. (Milan) 56(6), 424 (1974).
b) M. Kainosha and K. Ajisake, J. Am. Chem. Soc., 97, 5630 (1975).

3. H. Floss, E. Schleicher and R. Potts, J. Biol. Chem., 251, 5478 (1976).

4. C. Fuganti et al, J. Chem. Soc. Chem. Comm., 862 (1973).

5. W.J. Musliner and J.W. Gates Jr., J. Am. Chem. Soc., 88, 4271 (1966).

6. Y. Cheung and C. Walsh, J. Am. Chem. Soc., 98, 3397 (1976).

7. R. Hill and G. Newkome, J. Org. Chem., 34, 740 (1969).

8. Ethyl propiolate (9.7 g, 98.7 mmole) was dissolved in 25 g ethanol-d, 23 ml D_2O and 200 µl 30% NaOD. The yellow solution was stirred at 5°C for one hour and acidified with 1 M DCl. Extraction into CH_2Cl_2 followed by careful distillation yielded 7.4 g ethyl-[3-^{2}H]-propiolate (b.p. 120°) IR (neat) 2591 cm^{-1} (C-D), 1980 cm^{-1} (C≡C-D). Deuterium incorporation (by NMR) = 95%.

9. V. Martynov and I. Chou, Zhur, Obshcker. Khim., 30, 3174 (1960) [C.A. 55, 21042 c (1961)].

10. H. Bretschnieder, N. Karpitschka and G. Pierkarski, Mh. Chem., 84, 1084 (1953).

11. J. Greenstein and M. Winitz, "Chemistry of the Amino Acids", Wiley, New York, NY, 1961 pg. 2229.

12. H. Oqura, Y. Arata and S. Fujiwara, J. Mol. Spec., 23, 76 (1967).

13. D.J. Aberhardt, L. Lin and J.Y.R. Chu, J. Chem. Soc. Perkin I, 2517 (1975).

14. To affect high incorporation of deuterium, it is necessary to exhaustively pre-treat the catalyst with deuterium gas. This was accomplished by stirring the catalyst in ethyl acetate under deuterium and flushing the vessel several times with fresh gas over a period of 24 hours.

15. W.R. Vaughan and K.M. Milton, J. Am. Chem. Soc., 74, 5623 (1952).