SYNTHESES OF CHIRALLY LABELLED [4-^{13}C]-β-HYDROXYVALINE AND [4,4,4-$^{2}H_3$]-β-HYDROXYVALINE

D. John Aberhart
Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts 01545

SUMMARY

(E)-[4-^{13}C]-3-Methylcrotonic acid was converted to [4-^{13}C]-3-methyl-2,3-epoxybutyric acid, which was treated with ammonia to yield a mixture of [4-^{13}C]-β-hydroxyvaline and [4-^{13}C]-β-amino-α-hydroxyisovaleric acid, from which the β-hydroxyvaline of known chirality at C-3 relative to C-2 was isolated. Similarly, (E)-[4,4,4-$^{2}H_3$]-3-methylcrotonic acid was converted via the epoxide, into a mixture of [4,4,4-$^{2}H_3$]-β-hydroxyvaline and [4,4,4-$^{2}H_3$]-β-amino-α-hydroxyisovaleric acid, from which the β-hydroxyvaline was isolated.

Key Words: β-hydroxyvaline, β-amino-α-hydroxyisovaleric acid, deuterium, carbon 13.

INTRODUCTION

A recent paper in this journal (1) described the syntheses of β-hydroxyvaline and [4,4'-$^{2}H_6$]-β-hydroxyvaline. The authors suggested that routes proceeding via labelled β,β-dimethylacrylic acid or β,β-dimethylglycidic acid would not be suitable for the preparation of a variety of labeled β-hydroxyvalines. Their statements prompt me to report the syntheses of ^{13}C and ^{2}H labelled β-hydroxyvalines by a route which proceeds via labelled β,β-dimethylacrylic acid and β,β-dimethylglycidic acid.

RESULTS AND DISCUSSION

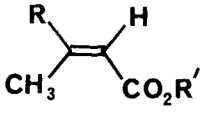
L-β-Hydroxyvaline occurs in nature as a constituent of the tetrapeptide designated P1, isolated from Cephalosporium sp. (2). It also is a constituent of the antibiotic berninamycin A (3) and of the antibiotics YA 56, X and Y (4),

0362-4803/83/050605-06$01.00

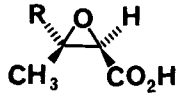
Received September 15, 1982
Revised December 22, 1982

isolated from Streptomyces species. The related β-hydroxy-N-methylvaline was recently found as a constituent of the antitumor antibiotics BBM-928 A, B, and C (5,6). Intermediates containing β-hydroxyvaline units have been suggested (7), but not proven (8), to be intermediates in penicillin biosynthesis.

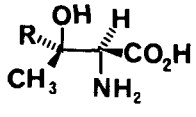
The objective of this work was the assignment of the ^{13}C and ^{1}H methyl signals of β-hydroxyvaline through the synthesis of β-hydroxyvaline labelled in one methyl group and having a known relative configuration at C-2 and C-3. (E)-[4-^{13}]-3-Methylcrotonic acid, 1a, was prepared by addition of lithium [^{13}C]-dimethylcuprate (ca. 15 atom % ^{13}C) to methyl tetrolate (9-11), followed by saponification of the resultant methyl (E)-[4-^{13}C]-3-methylcrotonate, 1b. The product, 1a, was converted by the method of Payne and Williams (12) to the racemic [4-^{13}C]-3-methyl-2,3-epoxybutyric acid, 2a (plus enantiomer). Treatment of the product with concentrated NH_4OH at 80°C essentially as previously reported (13) gave an approximately equal mixture of (2S,3S)-[4-^{13}C]-β-hydroxyvaline, 3a (plus enantiomer), and (2R,3R)-[4-^{13}C]-3-amino-2-hydroxyisovaleric acid, 4a (plus enantiomer). It has previously been shown (14) that such reactions proceed with clean anti epoxide opening. Recrystallization of the mixture led to the



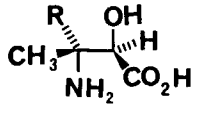
1 a R = $^{13}CH_3$, R′ = H
b R = $^{13}CH_3$, R′ = Me
c R = CD_3, R′ = H
d R = CD_3, R′ = Me



2 a R = $^{13}CH_3$
b R = CD_3



3 a R = $^{13}CH_3$
b R = CD_3



4 a R = $^{13}CH_3$
b R = CD_3

separation of pure 3a (plus enantiomer), albeit in rather low yield*. The product showed a ca. 15-fold enhanced intensity in the upfield ^{13}C methyl signal (δ 24.1) and normal intensity in the downfield methyl signal (δ 28.1, in D_2O. Pure 4a was not isolated.

In a similar manner, 1c (15,16) was converted to racemic 2b, which on treatment with NH_4OH gave a mixture of racemic 3b and 4b. The product mixture was directly crystallized to yield pure 3b (plus enantiomer) having a single ^{1}H methyl singlet at δ 1.32, whereas unlabeled β-hydroxyvaline has methyl singlets at δ 1.32 and δ1.51. Thus, although the yields are not high, the method is obviously suitable for the preparation of stereospecifically labelled β-hydroxyvaline. Also since both (E) and (Z)-methyl-labelled 3-methylcrotonic acids are readily synthesizable (1,15,16), and 3-methyl-2,3-epoxybutyric acid can be resolved (16), methyl-labelled β-hydroxyvaline of known absolute configuration at C-3 can be prepared by this method.

EXPERIMENTAL

General. ^{1}H NMR spectra taken on a Varian HA-100 instrument. ^{13}C NMR spectra were taken on a Bruker SXP 22/100 instrument at 22.63 MHz. Infrared specta were taken on a Perkin-Elmer 237 spectrometer. [^{13}C] and [$^2H_3$] Methyl iodide were obtained from Prochem and Merck, respectively. Tetrolic acid was obtained from Farchan Laboratories, Willoughby, Ohio.

Methyl (E)-[3-^{13}C]-3-methylcrotonate, 1b. [^{13}C]Iodomethane(2.0 g, 91 atom % ^{13}C) was mixed with unlabeled iodomethane (12.2 g), and the mixture in ether (25 ml) was added dropwise under N_2 (with refluxing and magnetic stirring) over 30 min to clean lithium wire (2.0 g, cut in ca. 150-200 pieces) in ether 30 ml. Refluxing and stirring was continued for 1 h. The mixture was allowed to stand undisturbed at 25°C overnight, and the clear supernatant (50 ml) was removed by double-tipped needle into a N_2-flushed bottle with rubber septum. The concentration, determined by titration with standard NaOH solution of an aliquot added to water was 1.47 M, 74% yield.

---

*A much better yield of 3a (and 3b) could be obtained by treatment of the mixture of 3 + 4 with benzoyl chloride/NaOH/H_2O followed by crystallization of the N-benzoate of 3 and acidic hydrolysis.

To a magnetically stirred suspension of cuprous iodide (5.72 g, 0.030 mole) in ether (60 ml) under N_2 at -20°C was added dropwise over 20 min the above $^{13}CH_3Li$ solution (1.47 M, 41 ml) to yield a pale brown solution. Stirring was continued at -20° for 1 h, then the temperature was lowered to -78°C, and dry THF (from $LiAlH_4$; 40 ml) was added. Then methyl tetrolate (2.94 g, 0.030 mole) (prepared from the acid with diazomethane, and kugelröhr distilled, bp 70-80°, 20 mm) in dry THF (20 ml) was added dropwise over 30 min. The resultant yellow suspension was stirred for an additional 2 h at -78°. Water (200 ml) was added over 15 min, and the mixture was filtered and the filtrate extracted with ether. The extract was washed with water and saturated brine, dried (Na_2SO_4), and evaporated to an oil, which was distilled in a kugelröhr tube at ca. 50°C, 20 mm, yielding 1b, 1.58 g; ir, ν_{max} ($CHCl_3$) 1725, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.90 (3H, s with a small amount, ca. 15%, of absorption appearing as a doublet, J = 126 Hz), 2.15 (s, 3), 3.63 (s, 3), 5.5 (br s, 1, $W_{1/2}$ = 6 Hz).

The deuterated analogue, methyl (E)-[3,3,3-$^2H_3$]-3-methylcrotonate, 1d, was prepared by a similar method, as briefly reported elsewhere (15); its ^1H NMR spectrum was the same as that of the unlabeled analogue except for the complete disappearance of the signal at δ 1.90.

(2S, 3S + 2R,3R)-[4-^{13}C]-β-Hydroxyvaline, 3a, plus enantiomer, and (2R,3R + 2S,3S)-[4-^{13}C]-3-Amino-2-hydroxyisovaleric Acid, 4a, plus enantiomer.

Methyl ester 1b (1.48 g, 0.013 mole) was treated with aqueous NaOH (1.0 M, 20 ml) at 33°C for 24 h. The resultant 1a was not isolated but was directly converted to 2a as follows. The pH of the solution was adjusted to 5.5 with 50% H_2SO_4; $Na_2WO_4 \cdot 2H_2O$ (0.64 g) was added, and the temperature was raised to 60-65°C. Then 30% H_2O_2 (2.5 ml) was added dropwise over 10 min, with maintenance of the temperature at 60-65°, and pH at 5.5 by addition of dilute NaOH as required. Heating at 60-65°C was continued for an additional 20 min, then the solution was cooled to 10°C, saturated with solid $(NH_4)_2SO_4$, and then acidified with conc. H_2O_4 to pH 2.0. The solution was rapidly extracted five times with ether, and the extract was dried (Na_2SO_4) and evaporated to yield crude 2a (800 mg). Since this product rapidly polymerized on standing at room temperature, it was used immediately in the next step. The crude product was dissolved in cold conc.

NH_4OH (15 ml), and then heated in a heavy-wall, sealed glass tube at 80°C for 16 h. After cooling, the solution was evaporated in vacuo. Recrystallization of the residue from acetone and a small amount of water gave [^{13}C]-β-hydroxyvaline, 3a, 25 mg, ^1H NMR (D_2O) δ 1.32 (ca. 2.5 H, s), 1.32 (ca. 0.5 H, d, J_{CH} = 128 Hz), 1.51 (3H, s), 3.68 (1H, s); ^{13}C NMR (D_2O) δ 24.1 (ca. 15X natural abundance), 28.1, 64.1, 70.6, 84.1, 172.8.

(2S,3S + 2R,3R)-[4,4,4-$^2H_3$]-β-Hydroxyvaline, 3b plus enantiomer, and (2R,3R + 2S,3S)-[4,4,4-$^2H_3$]-3-Amino-2-hydroxyisovaleric Acid, 4b plus enantiomer.

(E)-[4,4,4-$^2H_3$]-3-Methylcrotonic acid, 1c, 200 mg, prepared from 1d as previously reported (15,16), was converted by the above method into the crude racemic epoxy acid 2b, which was directly converted into a mixture of racemic 3b + 4b as described above. Recrystallization of the mixture from acetone-H_2O gave pure 3b, 20 mg, ^1H NMR (D_2O) δ 1.51 (3H, s), 3.68 (1H, s) (complete disappearance of the methyl signal at δ 1.32 appearing in the spectrum of unlabeled β-hydroxyvaline. Pure 4a or 4b could not be recovered from the above product mixtures by recrystallization.

ACKNOWLEDGMENT

This work was supported by grant GM 24420 from the National Institute of General Medical Sciences and by grant RR 05528 from the Division of Research Resources, National Institutes of Health. I thank Mr. Cliff McDonald for the ^1H NMR spectra, and Mr. Frank Shea for the ^{13}C NMR spectra recorded on an instrument supported, in part, by National Science Foundation equipment grant CHE-77-09059.

REFERENCES

1. Scott A.I. and Wilkinson T.J. - J. Labelled Comp. Radiopharm. 18: 347 (1981)
2. Loder P.B. and Abraham E.P. - Biochem. J. 128: 471 (1971)
3. Liesch J.M., Millington D.S., Pandey R.C., and Rinehart K.L. - J. Amer. Chem. Soc. 98: 8237 (1976)
4. Ito Y., Ohashi Y., Kawabe S., Abe H., and Okuda T. - J. Antibiotics (Japan) 25: 360 (1972)
5. Konishi M., Ohkuma H., Sakai F., Tsuno T., Koshiyama H., Naito T., and Kawaguchi H. - J. Amer. Chem. Soc. 103: 1241 (1981)

6. Arnold E. and Clardy J. - ibid 103: 1243 (1981)
7. Arnstein H.R.V. and Clubb M.E. - Biochem. J. 65: 18 (1957)
8. Aberhart D.J. - Tetrahedron 33: 1545 (1977)
9. Corey E.J. and Katzenellenbogen J.. - J. Amer. Chem. Soc. 91: 1851 (1969)
10. Siddall J.B., Biskup M., and Fried J.H. - ibid 91: 1853 (1969)
11. Hill R.K., Yan S., and Arfin S.M. - ibid 95: 7857 (1973)
12. Payne G.B. and Williams P.H. - J. Org. Chem. 24: 54 (1959)
13. Kaneko T. and Toshishige I. - Nippon Kagaku Zasshi 82: 743 (1961)
14. Harada K. and Oh-Hashi J. - Bull. Chem. Soc. Japan 39: 2311 (1966)
15. Aberhart D.J. - J. Org. Chem. 45: 5218 (1980)
16. Aberhart D.J. and Tann C.-H. - Bioorganic Chem. 10: 375 (1981)