Synthesis of Deuterium-labeled 1-Aminocyclopropane-1-carboxylic Acid

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SUMMARY

The syntheses of D,L-1-amino[2,2-$^2H_2$]cyclopropane-1-carboxylic acid;1-amino [c-2,c-3-$^2H_2$] and 1-amino[t-2,t-3-$^2H_2$]cyclopropane-1-carboxylic acid (cis-[$^2H_2$]ACC); 1-amino[2S,3S-$^2H_2$] and 1-amino[2R,3R-$^2H_2$]cyclopropane-1-carboxylic acid (trans-[$^2H_2$]ACC); and 1-amino[2,2,3,3-$^2H_4$]cyclopropane-1-carboxylic acid are described. The [2,2-$^2H_2$]and [2,2,3,3-$^2H_4$]ACC compounds were prepared from the appropriately deuterated 2-bromoethanol 4-methylbenzenesulfonates by reaction with ethyl isocyanoacetate and two moles of sodium hydride. The trans-[$^2H_2$]ACC and cis[$^2H_2$]ACC were prepared from meso and d,l-[1,2-$^2H_2$]-1,2-dibromoethane respectively, by reaction with ethyl isocyanoacetate and two moles of sodium hydride. These compounds are required for use in the study of the biosynthesis of ethylene by various plants and for use in ^1H and ^{13}C-NMR studies of 1-aminocyclopropane-1-carboxylic acid derivatives.

Key Words: Biosynthesis, Ethylene, Deuterium labeling,
1-Aminocyclopropane-1-carboxylic acid

INTRODUCTION

As part of our program to investigate the mechanisms of the enzymes responsible for the biosynthesis of the plant growth hormone ethylene from S-adenosyl-L-methionine, 1-aminocyclopropane-1-carboxylic acid synthase and the oxidative enzyme which converts ACC to ethylene, we required D,L-1-amino-[2,2-$^2H_2$]cyclopropane-1-carboxylic acid (D,L-[$^2H_2$]ACC); 1-amino[c-2,c-3-$^2H_2$]- and 1-amino[t-2,t-3-$^2H_2$]cyclopropane- 1-carboxylic acid (cis-[$^2H_2$]ACC); 1-amino-[2S,3S-$^2H_2$]- and 1-amino[2R,3R-$^2H_2$]cyclopropane-1-carboxylic acid (trans-[$^2H_2$]ACC); and finally 1-amino[2,2,3,3-$^2H_4$]cyclopropane-1-carboxylic acid ([$^2H_4$]ACC). These compounds are also currently being utilized to study the ^1H

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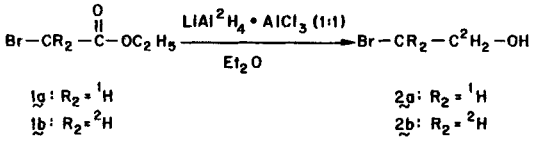
and ^{13}C-NMR spectra of various ACC derivatives.

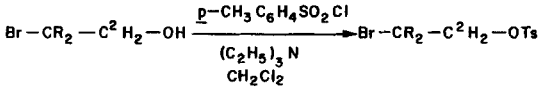
RESULTS AND DISCUSSION

Scheme I shows the synthesis of deuterated 2-bromoethanol 2a and 2b by the lithium aluminum deuteride-aluminum trichloride reduction (1) of ethyl bromoacetate 1a and ethyl 2-bromo[2,2-$^2H_2$]acetate 1b. The d,l-[1,2-$^2H_2$]-1,2-dibromoethane was prepared by bromination of cis-1,2-dideuterioethene with aqueous KBr_3 at 0°C in the dark by the method of Bernstein et al. (2). The deuterated 2-bromoethanol 4-methylbenzenesulfonates 3a and 3b were prepared from the deuterated 2-bromoethanol 2a and 2b via reaction with 4-methylbenzenesulfonyl chloride.

SCHEME I:



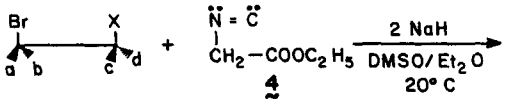
1g: $R_2 = {}^1H$ 2g: $R_2 = {}^1H$
1b: $R_2 = {}^2H$ 2b: $R_2 = {}^2H$



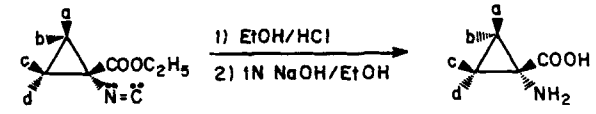
2a: $R_2 = {}^1H$ 3a: $R_2 = {}^1H$
2b: $R_2 = {}^2H$ 3b: $R_2 = {}^2H$

In addition to preparing the specifically-deuterated 1,2-dibromoethane 8 and 2-bromoethanol 4-methylbenzenesulfonates 3a and 3b, we also found it convenient to prepare our own ethyl isocyanoacetate due to its somewhat limited commercial availability. Ethyl isocyanoacetate 4 was prepared from N-formylglycine ethylester (3) via reaction with trichloromethyl chloroformate (liquid "diphosgene"). Liquid "diphosgene" is considerably safer and easier to use than phosgene (4) for the dehydration of the N-formyl group to an isonitrile group. In addition one can also prepare the dideuterated N-formyl glycine ethyl ester and dehydrate it to the dideuterated ethyl isocyanoacetate derivative. This compound can then be used as a synthon for the preparation of amino acids which are labeled with deuterium in the alpha position.

SCHEME II



	a	b	c	d	x
5:	^2H	^2H	^2H	^2H	OTs
6:	^1H	^1H	^2H	^2H	OTs
7:	^2H	^1H	^2H	^1H	Br
8:	^2H	^1H	^1H	^2H	Br
	^1H	^2H	^2H	^1H	Br



	a	b	c	d		a	b	c	d
9	^2H	^2H	^2H	^2H	13	^2H	^2H	^2H	^2H
10	^1H	^1H	^2H	^2H	14	^1H	^1H	^2H	^2H
	^2H	^2H	^1H	^1H		^2H	^2H	^1H	^1H
11	^2H	^1H	^2H	^1H	15	^2H	^1H	^2H	^1H
	^1H	^2H	^1H	^2H		^1H	^2H	^1H	^2H
12	^2H	^1H	^1H	^2H	16	^2H	^1H	^1H	^2H
	^1H	^2H	^2H	^1H		^1H	^2H	^2H	^1H
					17	^1H	^1H	^1H	^1H

The reaction sequences for the preparation of all the ACC's are shown in Scheme II. The route involves two Sn2-type displacements on appropriately deuterated ethane derivatives bearing leaving groups in the 1 and 2 positions by the *in situ* generated "disodium salt" of ethyl isocyanoacetate (5). The yields of the deuterated ethyl-1-isocyanocyclopropane-1-carboxylates from the deuterated 1,2-dibromoethanes were ~50% whereas the yields from the deuterated 2-bromoethanol 4-methylbenzenesulfonates were ~36%. These yields agree very closely with those obtained by Schollkopf *et al.*(5) for the undeuterated ethyl-1-isocyanocyclopropane-1-carboxylate which was 58% from 1,2-dibromoethane and 30% from 2-chloroethanol 4-methylbenzenesulfonate. The ethyl-1-isocyanocyclopropane-1-carboxylates were converted to the correspond-

ing ethyl-1-aminocyclopropane-1-carboxylates by ethanolic hydrogen chloride hydrolysis. These intermediates were then treated with ethanolic sodium hydroxide to give the desired ACC's.

The method reported in this paper for the synthesis of these regio-specifically deuterated ACC compounds represents a significant improvement over the previously published methods of Baldwin et al. (6,7) which utilize a lithio-derivative of benzylidene glycine ethyl ester. These authors reported the synthesis of [2,2,3,3-$^{2}H_{4}$]ACC in an overall yield of 28% (6) and the synthesis of cis- and trans-[$^{2}H_{2}$]ACC, which were contaminated with glycine in the amount of 22% and 49%, respectively(7), also in 28% yield. In our synthesis, there is no glycine contamination of the final ACC compounds. In addition, displacement of ethyl isocyanoacetate with the appropriately substituted dideuterio dibromoethanes gave a better cis:trans ratio of ACC derivatives than the displacement of the lithio derivative of benzylidene glycine ethyl ester.

EXPERIMENTAL

Melting points were determined on a Mel-Temp apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Brüker WH-360 MHz NMR spectrometer and on a Varian EM-360 60 MHz NMR spectrometer. Chemical shift values are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) in $CDCl_3$ and in parts per million (ppm) downfield from 3-(trimethylsilyl)tetradeuteriopropionate (TSP) in D_2O. cis 1,2[$^{2}H_{2}$]Ethene (96.5% ^{2}H), meso[1,2-$^{2}H_{2}$]-1,2-dibromoethane 5 (97.2% ^{2}H) and $LiAl^{2}H_{4}$ (98.9% ^{2}H) were purchased from Merck and Company. Trichloromethylchloroformate (liquid "diphosgene") was purchased from Fluka Chemical Corporation. Electron impact mass spectra were determined with a Finnigan 4021 Mass Spectrometer operating at an ionizing voltage of 70 eV., using direct probe insertion of the sample at 210 °C.

2-Bromo[1,1-$^2H_2$]ethanol 2a.

Compound 2a was prepared by a modification of the previously published procedure (1). A suspension of lithium aluminum deuteride (1.25 g ; 0.03 moles) in dry ether (150 ml) was stirred at -78°C for 30 min. To the cooled, stirred suspension, anhydrous aluminum chloride (4.00g, 0.03 moles) was added in small portions from a solid addition device in a manner that allowed the temperature of the stirred suspension to be maintained at ~ -75°C. The mixture was then stirred for an additional 30 min at -78°C. To this suspension, ethyl bromoacetate (5.0g, 0.03 moles) in 50 ml of dry ether was added dropwise over a 30 min period from a constant pressure addition funnel. The resultant mixture was then stirred at -78°C for an additional 2hr and then allowed to warm slowly to room temperature. Excess deuteride was destroyed by the addition of ethyl acetate (5 ml) followed by water (5 ml). The ether solution was decanted and the aluminum salts were washed with ether (3 x 75ml). The combined ether extracts were dried (Na_2SO_4), concentrated and distilled to give 2.2g(58%) of 2-bromo[1,1,$^2H_2$]ethanol 2a bp 56-57°C/20mm Lit(1) 65-68°C/33 mm ^{1}H NMR ($CDCl_3$)δ 2.52(S, 1H, OH), 3.64(S,2H,-CH_2Br).

2-Bromo[1,1,2,2-$^2H_4$]ethanol 2b.

Compound 2b was prepared by the same procedure described for the preparation of 2-bromo[1,1$^2H_2$]ethanol 2a. Ethyl 2-bromo[2,2-$^2H_2$]acetate(1) (8.45g, 0.05 moles) and lithium aluminum deuteride (2.01g, 0.05 moles) anhydrous aluminum chloride (6.6g, 0.05 moles) were reacted to give 3.35g (52%) of 2-bromo[1,1,2,2-$^2H_4$]ethanol 2b bp 56-57°C/20mm.

2-Bromo[1,1-$^2H_2$]ethanol 4-methylbenzenesulfonate 3a.

To a solution of compound 2a (2.0g, 0.0158 moles) and triethylamine (1.72g, 0.017 moles) in dry dichloromethane (10 ml) at 0°C was added with stirring 4-methylbenzenesulfonyl chloride (3.43g, 0.018 moles). The reaction

mixture was stirred at 0°C. (2 hr) and room temperature overnight. The triethylamine hydrochloride was filtered and the filtrate was taken up in ether (100ml). The organic layer was washed with water and dried (Na_2SO_4).

The solvent was removed on a rotary evaporator to yield 4.0g (90%) of tosylate 3a. Lit.(8) mp. 11.8°C for the nondeuterated analog. ^1H NMR($CDCl_3$)δ2.44-(S,3H,CH_3), 3.48(S,2H-CH_2Br) 7.28 - 7.98 (m.4H,ArH).

2-Bromo[1,1,2,2-$^2H_4$]ethanol 4-methylbenzenesulfonate 3b.

Compound 3b was prepared from 2b according to the procedure described for the preparation of 2-bromo[1,1-$^2H_2$]ethanol 4-methylbenzenesulfonate 3a: yield 93% ^1H NMR($CDCl_3$)δ2.41(s,3H,-CH_3), 7.32-8.12(m,4H,ArH).

Ethyl isocyanoacetate 4.

To a boiling solution of N-formylglycine ethyl ester (3) (19.6g, 0.15 moles) and triethylamine (120ml) in dry dichloromethane (200ml) was added dropwise trichloromethyl chloroformate ("diphosgene") (4) (19.3 ml, 31.8g, 0.16 moles) in dichloromethane (50 ml) over a period of 2 hr. After refluxing for an additional 30 min, the cooled mixture was poured onto crushed ice and extracted with ether (3 x 150 ml). The combined organic extracts were washed with 10% Na_2CO_3 (3 x 100 ml); water; dried (Na_2SO_4); and evaporated. The obtained dark brown liquid was distilled under vacuum to give 9.5 g (56%) of the product as a colorless liquid. bp 73-74°C/3mm Lit.(4) 76-78°C/4mm. ^1H NMR ($CDCl_3$) δ 1.32 (t,3H,J=7.2Hz,-CH_2CH_3), 4.18(s,2H,-CH_2-), 4.24(q,2H,J=7.2Hz,-CH_2CH_3).

D,L-1-amino[2,2-$^2H_2$]cyclopropane-1-carboxylic acid 14.

Sodium hydride (1.4g of 60% dispersion in mineral oil, 0.037 moles) was added in small portions to a solution of ethyl isocyanoacetate 4 (1.87g, 0.0175 moles), 3a (4.92g 0.0175 moles) and dry dimethyl sulfoxide (19 ml) in

75 ml of dry ether at 20°C in a flame-dried flask under nitrogen. After being stirred at 20°C for 1 hr, the reaction mixture was allowed to warm to room temperature and then heated to reflux for 30 min. The mixture was poured into water and extracted with ether (5 x 50 ml). The organic layer was washed with water (3 x 100 ml), dried (Na_2SO_4) and concentrated under water aspirator pressure. The residue was distilled to give 10, 0.85g (36% yield) bp 67-69°C/2.5 mm. ^{1}H NMR ($CDCl_3$) δ 1.33(t,3H,J=7.2Hz,-$CH_2\underline{CH}_3$), 1.53 and 1.63 (d,2H,J=4.9Hz,-$C\underline{H}_2$-), 4.26(q,2H,J=7.1Hz,-$C\underline{H}_2CH_3$).

A solution of the isonitrile 10 (0.5g, 0.0035 moles) in ethanol (2 ml) was added dropwise to a saturated solution of hydrogen chloride in ethanol (8 ml) at -10°C and stirred at room temperature for 24 hr. After removal of the solvent in vacuo, the residue was dissolved in 1N ethanolic sodium hydroxide (10 ml) and stirred at room temperature for 1 hr. The reaction mixture was then acidified with 2N HCl and the solvent was evaporated in vacuo. The residue was dissolved in water (3 ml) and applied to a column of Dowex 50X W4-200 (NH_4^+ 100 ml) and eluted with water followed by 1N ammonium hydroxide. The fractions containing the product 14 were concentrated in vacuo and lyophilized. Yield 0.34g (93%) mp 242-244°C ^{1}H NMR (D_2O) δ 1.05 and 1.15 (d,2H,J=5.8Hz). MS, m/e (relative intensity) 104 (MH^+, 20.1). The isotopic purity of the compound 14 was determined by mass spectrometry to be 92% d_2.

1-Amino[2,2,3,3-$^2H_4$]cyclopropane-1-carboxylic acid 13.

According to the procedure described for isonitrile 10, 1.4g (0.0124 moles) of ethyl isocyanoacetate 4, 3.7g (0.013 moles) of 2-bromo[1,1,2,2-$^2H_4$]ethanol 4-methylbenzenesulfonate 5 and 1.12g (0.028 moles) of sodium hydride (60% dispersion in mineral oil) were reacted to give 0.71g (38%) of isonitrile 9 bp 68-70°C/2.5 mm ^{1}H NMR ($CDCl_3$) δ 1.33 (t,3H, J=7.1Hz,-$CH_2\underline{CH}_3$), 4.26(q,2H,J=7.1Hz,-$C\underline{H}_2CH_3$).

[2,2,3,3-$^2H_4$]ACC 13 was prepared in 94% yield from isonitrile 9 by the procedure described for the preparation of compound 14. MS, m/e (relative intensity) 106 (MH^+, 61.8). The isotopic purity of the compound 13 was determined by mass spectrometry to be 99.8% d_4.

1-Amino[cis-$^2H_2$]cyclopropane-1-carboxylic acid 15.

From 3.0g of meso-1,2-dibromo[1,2-$^2H_2$]ethane 7, there was obtained 1.02g (48%) of isonitrile 11 (as a 1:1 mixture) in a procedure similar to that used for the preparation of 10. bp 50-52°C/1 mm Lit(5) bp 90-93°C/15mm fo the nondeuterio analog. ^1H NMR ($CDCl_3$) δ 1.30 (t,3H,J=7.2Hz, $-CH_2CH_3$), 1.51 (S,1H), 1.60 (S,1H), 4.23 (q,2H,J=7.2Hz,$-CH_2CH_3$). Compound 15 (as a 1:1 mixture) was prepared in 90% yield from 11 by following the procedure for 14 mp 242-244°C (recrystallized ethanol/water). ^1H NMR (D_2O) δ 1.0 (s,1H), 1.15 (s,1H). MS, m/e (relative intensity) 104 (MH^+, 7.0). The isotopic purity of the compound 15 was determined by mass spectrometry to be 80% d_2.

1-Amino[trans-$^2H_2$]cyclopropane-1-carboxylic acid 16.

Isonitrile 12 was prepared in 52% yield from (d,ℓ)-1,2-dibromo [1,2-$^2H_2$]ethane 8 by following the procedure for the synthesis of isonitrile 10. bp 58-59°C/1.3mm. ^1H NMR: ($CDCl_3$) δ 1.33(t,2H, J=7.2Hz,$-CH_2CH_3$), 1.54(d,J=8.4Hz,1H), 1.63(d,J=8.4Hz,1H), 4.26(q,2H, J=7.2Hz, $-CH_2CH_3$).

The procedures for obtaining 14 from 10 were applied to isonitrile 12 affording 16: 95% yield mp 242-244°C. ^1H NMR: (D_2O) δ 1.0(d, J=7.6Hz,1H), 1.15(d,,J=7.6Hz,1H). MS, m/e (relative intensity) 104 (MH^+, 47.0). The isotopic purity of the compound 16 was determined by mass spectrometry to be 96% d_2.

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