Synthesis of 1-Aminocyclopropenecarboxylic Acid: The Unsaturated Analogue of ACC

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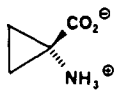
The unsaturated analogue of ACC (1-aminocyclopropenecarboxylic acid), the biosynthetic precursor in plants to ethylene, has been synthesized in nine steps from commercially available bis(trimethylsilyl)acetylene. Key steps in the synthesis are a Curtius rearrangement of 1-(methoxycarbonyl)-2-cyclopropenecarbonyl azide to the corresponding isocyanate, conversion of the isocyanate to 1-[(*tert*-butoxycarbonyl)amino]-2-cyclopropenecarboxylic acid, and hydrolysis in 5% HCl to the hydrochloride salt of 1-aminocyclopropenecarboxylic acid (**11**). The novel amino acid **11** has shown potent inhibition of ethylene biosynthesis in soybean leaf disk assays.

In the last 10 years considerable effort has been devoted to the synthesis and study of 1-aminocyclopropenecarboxylic acids, 10 of which have been isolated from microorganisms and higher plants.[1] In 1979[2] the parent 1-aminocyclopropanecarboxylic acid (ACC, **1**) was reported to be the immediate precursor in plant biosynthesis of ethylene, an important phytohormone that initiates fruit ripening and regulates many aspects of plant growth.[3] Few details concerning the mechanism by which the ethylene-forming enzyme (EFE) converts ACC to ethylene are known.[4]



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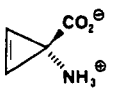

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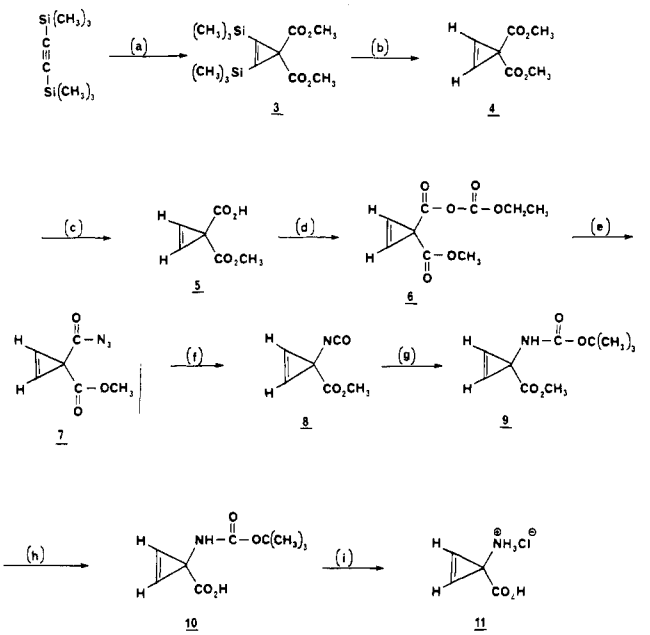
It occurred to us that the cyclopropene analogue of ACC, **2**, if recognized by the EFE, might serve as an excellent inhibitor of ethylene biosynthesis. If **2** is successfully operated upon by the EFE, the product will be acetylene, which has only weak plant hormone properties. It is also possible that reactive intermediates formed by the action of EFE on **2** might react irreversibly with the EFE in an enzyme suicide substrate fashion. In either case, **2** should be a good inhibitor of ethylene biosynthesis. A potent inhibitor of ethylene formation could have important commercial utility in retarding fruit ripening and spoilage, delaying the senescence of plants, and enhancing crop yields.

In this paper we report the synthesis of **2** (isolated as its hydrochloride salt) in nine steps starting from commercially available bis(trimethylsilyl)acetylene.

Our strategy for the synthesis of **2** began with an examination of the routes used to prepare 1-aminocyclopropanecarboxylic acids. ACC analogues have been prepared by the cyclopropanation of derivatives of α,β-dehydroamino acids,[5] by the cycloalkylation of glycine anion equivalents,[6] by the Curtius rearrangement of acyl azides generated from cyclopropane-1,1-dicarboxylates,[7] by the addition of aminocarboxycarbene to alkenes,[8] and by the α-nitration of cyclopropanecarboxylic acid.[9] Of these five approaches, only the Curtius rearrangement would appear to afford promise for the synthesis of the cyclopropene analogue of ACC.

The route used for the synthesis of **2** (as the hydrochloride salt **11**) is shown in Scheme I.

Scheme I[a]



[a] (a) Reagents and conditions: (a) $N_2C(CO_2CH_3)_2$, $Cu(acac)_2$, 33%; (b) 10% K_2CO_3, THF–H_2O, 85%; (c) NaOH, CH_3OH–H_2O, 84%; (d) K_2CO_3, $ClCO_2CH_2CH_3$, THF, dicyclohexano-18-crown-6 ether; (e) NaN_3, THF–H_2O; (f) Δ, benzene; (g) Δ, t-C_4H_9OH, 32% (from **5**); (h) NaOH, CH_3OH–H_2O, 84%, (i) 5% HCl, rt, 95%.

A recent report[10] describes the synthesis of **4** in 11% yield. The yield of **3** was increased from the reported

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10–13% to >30% by using cupric acetylacetonate as the catalyst[11] and adding the diazomalonate at a metered rate. Compound **3** was smoothly desilylated to **4** by stirring at room temperature in 2:1 THF–H_2O containing 10% K_2CO_3. Yields of 85–95% of **4** were obtained, and the purification of this material by flash chromatography was considerably easier than for that produced by the literature method.

Hydrolysis of one of the two ester moieties in **4** to give the monoacid **5** was accomplished by stirring **4** with 1 equiv of NaOH in alcohol–water at room temperature. The alcoholic solvent used (in this case methanol) in the saponification must be the conjugate acid of the alkoxy function of the ester moieties. Thus, when ethanol was used in the saponification of **4**, rapid but incomplete transesterification occurred, and a complex mixture of ester–acid products was produced. The NMR spectrum of the isolated monoacid **5** gave no evidence that any bisacid contaminant was present.

It was possible to convert **3** directly to the monoacid **5** with 1.0 equiv of NaOH in CH_3OH–H_2O. However, the acid resulting from this one-step procedure, although giving the expected NMR spectrum, contained a yellow impurity that could not be removed by recrystallization.

Initial attempts at converting **5** to the mixed anhydride **6** employed conditions (triethylamine–ethyl chloroformate–acetone, 0 °C) that had given excellent yields of a stable mixed anhydride when applied to 1-(ethoxycarbonyl)-1-cyclopropanecarboxylic acid. Under these conditions, **5** appeared (from the infrared spectrum of an aliquot) to be converted to **6**, but **6** decomposed rapidly. We concluded that this decomposition was being induced by the triethylamine hydrochloride, but removal of the amine hydrochloride by precipitation with cold ether and filtration did not prevent decomposition of the mixed anhydride.

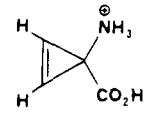
The mixed anhydride **6** was synthesized by stirring the acid **5** with ethyl chloroformate, anhydrous K_2CO_3, THF, and dicyclohexano-18-crown-6 ether. If the methyl ester **5** is used, the reaction must be run at room temperature for **6** to form. The ethyl ester corresponding to **5** is completely converted to the mixed anhydride in ~2 h at 0 °C. The reaction is monitored in the IR, and the reaction is regarded as complete when the 1820-cm^{-1} (anhydride) absorption is nearly equivalent in intensity to the 1740-cm^{-1} (ester) absorption. No isolation or further characterization of the mixed anhydride was attempted.

After the K_2CO_3 was removed by filtration, the THF solution of **6** was converted to the carbonyl azide **7** by stirring at 0 °C with sodium azide in a minimum volume of water. The azide **7** was converted to the isocyanate **8** by heating at reflux in benzene.

The isocyanate **8** was converted to the *tert*-butyl carbamate **9** by heating at reflux for several hours in dry *tert*-butyl alcohol. The *tert*-butyl carbamate **9** could also be prepared without isolation of the intermediate isocyanate by heating the azide **7** at reflux for several hours in dry *tert*-butyl alcohol. The carboxylic acid **10** was prepared in good yield by stirring **9** with 1.2 equiv of NaOH in methanol–water solution for 2 days at ambient temperature.

When **10** was stirred for 6 h at room temperature in dichloromethane containing a few drops of TFA,[12] it was recovered unchanged. However, **10** was converted to the amine hydrochloride salt of 1-aminocyclopropenecarboxylic acid (**11**) in 95% by stirring with 5% HCl at room temperature.

The proton NMR, ^{13}C NMR, infrared, and FAB mass spectra all support the proposed structure for **11**. In D_2O, the proton NMR of **11** shows a single sharp absorption for the two olefinic protons at δ 7.5. The ^{13}C NMR in D_2O shows peaks at δ 37.12 (quaternary C), δ 108.17 (olefinic C's), and δ 174.29 (acid carbonyl). (^{13}C signals for the tetrahedral and acid carbonyl in ACC come at δ 36.28 and 176.43, respectively). The FAB positive-ion spectrum shows a base peak at mass 100, which may be assigned the structure **13**, and the FAB negative ion spectrum shows a peak at mass 98 assignable to **14**.



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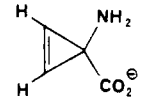

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We have used the route shown in Scheme I to synthesize multigram quantities of **11**. However, this route cannot be successfully used for the synthesis of analogues of **11** bearing alkyl substituents on the cyclopropene ring. For example, treatment of 1-[(*tert*-butoxycarbonyl)amino]-2,3-dimethylcyclopropene-1-carboxylic acid with 5% HCl at room temperature gives a complex mixture of products arising from electrophilic attack on the cyclopropene ring.

The cyclopropene analogue of ACC, **11**, has demonstrated potent inhibition of ethylene formation in soybean leaf disk assays.[13] Acetylene was observed in trace amounts by gas chromatographic methods in the assays where **11** was present.

Experimental Section

General Methods. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. ^1H NMR spectra (Me_4Si internal standard) were recorded on a 60-MHz Varian EM360L spectrometer. Carbon NMR were obtained at 22.5 MHz on a JEOL FX-90Q spectrometer. Infrared spectra were obtained on a Perkin-Elmer Model 299B. The FAB mass spectra were obtained on a VG-7070 mass spectrometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

Dimethyl 2,3-Bis(trimethylsilyl)cyclopropene-1,1-dicarboxylate (3). To a mixture of 165.2 g (0.970 mol) of bis(trimethylsilyl)acetylene and 0.500 g (1.91 mmol) of cupric acetylacetonate heated at 145 °C under an atmosphere of N_2 was added 30.1 g (0.190 mol) of dimethyl diazomalonate injected at the rate of 1.0 mL/h by means of a syringe pump. Progress of the reaction was monitored by IR. At the end of 36 h, no diazo absorption (2130 cm^{-1}) was present. The excess bis(trimethylsilyl)acetylene was distilled off under reduced pressure to leave a dark amber-colored residue, which was purified by flash chromatography on silica gel (80:20 hexane–ethyl acetate) to give 19.2 g (33%) of **3** as a light yellow oil: ^1H NMR ($CDCl_3$) δ 0.20 (s, 18 H), 3.60 (s, 6 H).[14]

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(9) Haner, R.; Seebach, D. *Chimia* **1985**, *39*, 356.

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(11) When rhodium(II) acetate dimer was used as the catalyst, none of the desired **3** was obtained. The use of trimethyl phosphite–cuprous iodide as the catalyst gave results comparable with those obtained with cupric acetylacetonate.

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Dimethyl Cyclopropene-1,1-dicarboxylate (4). A solution of 18.7 g (0.0622 mol) of **3** in 190 mL of THF was cooled to 0 °C, and 95 mL of a 10% K_2CO_3 solution in water was added dropwise. The mixture was allowed to warm to room temperature, stirred 2 h, poured into ice water (150 mL), extracted with ether (3 × 150 mL), and dried ($MgSO_4$), and the ether was removed to leave an orange oil. Purification was by flash chromatography through silica gel (75:25 hexane–ethyl acetate) to give 8.3 g (85%) of **4** as a slightly yellow oil: ^1H NMR ($CDCl_3$) δ 3.72 (s, 6 H), 6.91 (s, 2 H).[15]

1-(Methoxycarbonyl)-2-cyclopropenecarboxylic Acid (5). A solution of 8.2 g (52.0 mmol) of **4** in 80 mL of methanol was cooled to 0 °C, and a solution of 2.10 g (52.5 mmol) of NaOH in 8.0 mL of water was added dropwise. The mixture was allowed to warm to room temperature and stirred for 2 days. The mixture was concentrated under reduced pressure, poured into ice water (250 mL), extracted with ether (2 × 100 mL), acidified with 10% HCl, and extracted with ethyl acetate (3 × 100 mL). The ethyl acetate solution was dried ($MgSO_4$) and the solvent removed to give 6.3 g (84%) of **5** as a pale yellow oil, which solidified under vacuum: mp 53–55 °C; ^1H NMR ($CDCl_3$) δ 3.80 (s, 3 H), 6.90 (s, 2 H), 11.62 (s br, 1 H); IR (CH_2Cl_2) 1760, 1710, 1670 cm^{-1}. Anal. Calcd. for $C_6H_8O_4$: C, 50.00; H, 5.60. Found: C, 50.09; H, 5.49.

Methyl 1-[(*tert*-Butoxycarbonyl)amino]cyclopropenecarboxylate (9). A solution of 5.0 g (35.2 mmol) of **5** in 50 mL of THF was cooled to 0 °C, and 9.7 g (70 mmol) of anhydrous K_2CO_3 and 1.0 g (2.7 mmol) of dicyclohexano-18-crown-6 ether were added. To this mixture was added dropwise a solution of 4.20 g (38.7 mmol) of ethyl chloroformate in 10 mL of THF. After the mixture was stirred for 30 min at 0 °C, an IR of the mixture showed only a weak absorption for the mixed anhydride at 1820 cm^{-1}. The reaction mixture was allowed to come to room temperature and stirred 2 h; an additional 0.50 g (1.35 mmol) of the crown ether was added, and the mixture was stirred an additional 30 min at room temperature. An IR of the reaction mixture at this point showed an 1820-cm^{-1} absorption nearly equal in intensity to that of the ester carbonyl at 1740 cm^{-1}.

The K_2CO_3 was removed from the mixture by rapid suction filtration. The filtrate was returned to the reaction flask and cooled to 0 °C, and a solution of 2.52 g (38.7 mmol) of NaN_3 in 20 mL of water was added rapidly. After being stirred for 30 min at 0 °C, the mixture was poured into 250 mL of ice water and extracted with ether (3 × 150 mL). The ether solution was dried ($MgSO_4$) and the solvent removed to leave ~6.0 g of the acid azide **7** as a red oil: IR (CH_2Cl_2) 2140, 1740 cm^{-1}. This material was taken up in 70 mL of dry benzene and heated at reflux under N_2 for 30 min. Removal of the benzene under reduced pressure left ~5.0 g of the cyclopropenyl isocyanate **8** as a red oil: IR (CH_2Cl_2) 2240, 1735 cm^{-1}. The isocyanate was taken up in 70 mL of dry *tert*-butyl alcohol and heated at reflux under N_2 for 6 h. The excess *tert*-butyl alcohol was removed under reduced pressure and the residue was taken up in 100 mL of ethyl acetate and filtered to remove 0.55 g of a white solid (mp 216–217 °C), which was determined to be *N,N'*-bis(1-(methoxycarbonyl)-2-cyclopropenyl)urea: IR (CH_2Cl_2) 3320, 1740, 1635 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.48 (s, 6 H), 6.60 (s br, 2 H), 7.60 (s, 4 H). Anal. Calcd for $C_{11}H_{12}N_2O_5$: C, 52.38; H, 4.80; N, 11.11. Found: C, 52.46; H, 4.84; N, 11.23.

The ethyl acetate was removed from the filtrate under reduced pressure, and the residue was purified by flash chromatography on silica gel (75:25 hexane–ethyl acetate) to give 2.4 g (32% from **5**) of **9** as a light yellow solid: mp 83.0–84.5 °C; IR (CH_2Cl_2) 3440, 1730, 1660, 1485, 1370, 1260–1240, 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (s, 9 H), 3.70 (s, 3 H), 5.53 (s br, 1 H); 7.27 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 28.387 (q, *tert*-butyl CH_3), 35.457 (s, cyclopropene quaternary C), 52.522 (q, ester CH_3), 79.660 (s, *tert*-butyl quaternary C), 108.565 (d, olefinic C), 155.534 (s, carbamate C=O), 173.818 (s, ester C=O). Anal. Calcd for $C_{10}H_{15}NO_4$: C, 56.33; H, 7.09; N, 6.57. Found: C, 56.16; H, 6.95; N, 6.42.

1-[(*tert*-Butoxycarbonyl)amino]-2-cyclopropenecarboxylic Acid (10). A solution of 2.37 g (11.1 mmol) of **9** in 50 mL of methanol was cooled to 0 °C, and 0.53 g (13.3 mmol) of NaOH in 10 mL of water was added dropwise. The mixture was allowed to come to room temperature and stirred 2 days. The mixture was concentrated under vacuum, and the residue was diluted with ice water (150 mL), extracted with ether (2 × 75 mL), and acidified with 10% HCl. The acidic mixture was extracted with ethyl acetate (3 × 100 mL), the ethyl acetate solution dried ($MgSO_4$), and the solvent removed to leave a light yellow solid. This was recrystallized from hexane–ethyl acetate to give 1.85 g (84%) of **10** as a white solid: mp 139 °C dec; IR (CH_2Cl_2) 2900–2000, 1710, 1480, 1420, 1370, 1270, 1245, 1160 cm^{-1}; ^1H NMR (acetone-d_6) δ 1.48 (s, 9 H), 5.90 (s br, 2 H, $CO_2H \cdot {}^1/_2H_2O$), 7.57 (s, 2 H); ^{13}C NMR (acetone-d_6) δ 28.36 (*tert*-butyl CH_3), 35.26 (cyclopropene quaternary C), 78.88 (*tert*-butyl quaternary C), 109.89 (cyclopropene olefinic), 156.37 (carbamate C=O), 175.39 (acid C=O). Anal. Calcd for $C_9H_{13}NO_4$: C, 54.26; H, 6.58; N, 7.03. Found: C, 53.68; H, 6.70; N, 6.72.

1-Aminocyclopropenecarboxylic Acid Hydrochloride (11). A suspension of 1.80 g (9.00 mmol) of **10** in 200 mL of 5% aqueous HCl was stirred for 4 h at room temperature. The suspension of **10** slowly dissolved, and gas evolution was observed. The solution was evaporated under reduced pressure to leave a white solid, which was triturated 3× with 50 mL of ether and dried overnight at 40 °C in a vacuum oven to give 1.16 g (95%) of **11** as a white, crystalline solid: mp 164 °C dec; IR (KBr) 3500–2300, 1735, 1660, 1480, 1305, 1210, 1095, 1010, 885 cm^{-1}; ^1H NMR (D_2O) δ 7.50 (s); ^{13}C NMR (D_2O) δ 37.12 (s, cyclopropene quaternary C), 108.17 (d, olefinic C's), 174.29 (s, acid C=O); FAB mass spectrum (positive ions, thioglycerol matrix) major peak m/z 98.0. Anal. Calcd for $C_4H_6ClNO_2 \cdot H_2O$: C, 31.28; H, 5.25; N, 9.11; Found: C, 31.20; H, 5.23; N, 9.07.

Acknowledgment. We are grateful to Dr. J. D. Holmsen for testing these materials for inhibition of ethylene biosynthesis and to Dr. J. A. Durden for several constructive suggestions made in the course of this synthetic work.

Registry No. **3**, 102127-45-7; **4**, 102127-47-9; **5**, 102137-67-7; **6**, 110374-48-6; **7**, 110374-49-7; **8**, 110374-50-0; **9**, 110374-51-1; **10**, 110374-52-2; **11**, 110374-53-3; **11** (base), 110374-54-4; *N,N'*-bis-(1-(methoxycarbonyl)-2-cyclopropenyl)urea, 110391-32-7; bis-(trimethylsilyl)acetylene, 14630-40-1; dimethyl diazomalonate, 6773-29-1.

(14) Literature[10] δ are 0.25 and 3.678, respectively.
(15) Literature[10] δ are 3.76 and 6.90, respectively.