Ethylene Biosynthesis. 6. Synthesis and Evaluation of Methylaminocyclopropanecarboxylic Acid

Michael C. Pirrung* and Gerard M. McGeehan

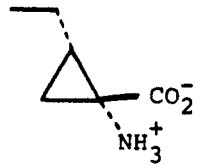
Department of Chemistry, Stanford University, Stanford, California 94305

Received September 6, 1985

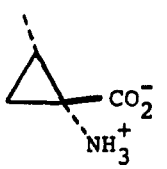
The compound (1*R*,2*S*)-2-methyl-1-aminocyclopropanecarboxylic acid (MeACC) has been synthesized in racemic and optically active forms. It has been studied as a substrate and inhibitor for ethylene biosynthesis in mung bean hypocotyls. Both K_I and K_m for MeACC are the same and identical with K_m for ACC, strongly implying that they compete for the same site. MeACC is converted to propene by mung bean hypocotyls.

The biosynthesis of ethylene is a problem that has demanded attention in plant physiology almost from the moment of the discovery that ethylene is a hormone. Historically,[1-7] research in this area has been punctuated by leaps of insight and discovery, such as Burg's proposal[8] that the activity of ethylene and alkene analogues could be related to their binding affinity to a transition metal and Yang's finding that 1-aminocyclopropanecarboxylic acid (ACC) is the immediate biosynthetic precursor to ethylene.[9] The novelty of Yang's discovery has not yet allowed much opportunity for the development of ACC analogues; a wealth of commercially available compounds have little to no activity.[10-12]

One prominent exception to the foregoing is (+)-allocoronamic acid (**1**), a compound studied by Ichihara, Yang, and co-workers.[13] They found that of the four possible stereoisomers of ethyl ACC, only **1** is processed to a major



1



2

extent (>40:1) by plant tissue, and it is converted to 1-butene. While the rate relative to ACC (0.25 in mung bean) and competitive inhibition were mentioned, the compound's action as an analogue was not well-characterized kinetically. An active site model was proposed which accommodates its stereochemistry, which was assigned by NMR and ORD data.

Recent synthetic endeavors toward alkylated ACC analogues have been conducted by Walsh and Baldwin. The racemic vinyl, methyl, and ethyl compounds, the latter in deuterated form, were prepared by Walsh.[14] The key intermediate in these syntheses is a cyclopropyl malonate which is selectively ammonolyzed at its less-hindered carbonyl group to provide an amide which is subjected to Hoffman degradation. This route ultimately affords analogues with syn stereochemistry of the alkyl substituent relative to the carboxyl. These compounds were used in the studies of the *Pseudomonas* enzyme ACC-deaminase which, interestingly, has stereochemical requirements opposite those for ethylene biosynthesis. Baldwin[15] has recently modified Walsh's route to provide the correct relative stereochemistry for methyl and ethyl analogues in ethylene biosynthesis. This involves first hydrolyzing the cyclopropyl malonate and then executing an ammonolysis/Curtius degradation sequence. Baldwin has also prepared deuterated analogues using this methodology and has resolved and assigned absolute configurations to these products. This paper reports a much more direct method for preparing those analogues which are useful for ethylene biosynthesis studies. It includes the first synthesis of such compounds from materials of unambiguous absolute configuration and provides hard evidence concerning the steric requirements of the active center for ethylene production.

Experimental Section

Mung bean hypocotyl segments were obtained by the procedure of Yang.[16] Incubation experiments were conducted in 25-mL Erlenmeyer flasks sealed with rubber serum stoppers and containing 10 or 20 segments in 2 mL of solution (50 mM morpholine–ethanesulfonic acid, pH 6.1, 2% sucrose, 50 μg/mL chloramphenicol). Gas samples (2 mL) were periodically withdrawn with a gas-tight syringe and analyzed by gas chromatography[17] using digital integration. The integrator was calibrated with an external standard of ethylene or propylene in helium. Analysis was conducted at 40 °C (C_2H_4) or 90 °C (C_3H_6). Optimum conditions on a Perkin-Elmer Sigma 3B gas chromatograph were injector, 125 °C; detector, 140 °C. Rates were determined by a linear least-squares fit with fair precision ($r > 0.95$).

(1*R,2*S**)-Ethyl 2-Methyl-1-isocyanocyclopropanecarboxylate.** The procedure of Schollkopf[18] was followed. To a suspension of pentane-washed sodium hydride (1.93 g of 60% suspension, 48.3 mmol) in 20 mL of ether in a 250-mL, 3-necked, round-bottomed flask was added over 1 h by a dropping funnel ethyl isocyanoacetate (Aldrich, redistilled) (2.45 g, 21.7 mmol) and 1,2-dibromopropane (4.43 g, 21.9 mmol) in a solution of 25 mL of Me_2SO and 60 mL of ether. The funnel was rinsed with an additional 10 mL of ether and was replaced by a reflux condenser. After 2 h at reflux, the reaction mixture, now dark, was cooled and poured into 50 g of an ice/water mixture overlayed with 50 mL of ether. After gas evolution subsided, the aqueous layer was used to dissolve and transfer salts from the reaction

(1) Bate, J. B. *Annu. Rev. Plant Physiol.* **1950**, *1*, 183-206.
(2) Ulrich, R. *Annu. Rev. Plant Physiol.* **1950**, *9*, 385-416.
(3) Burg, S. P. *Annu. Rev. Plant Physiol.* **1962**, *13*, 265-302.
(4) Abeles, F. B. *Annu. Rev. Plant Physiol.* **1972**, *23*, 259-292.
(5) Lieberman, M. *Annu. Rev. Plant Physiol.* **1979**, *30*, 533-591.
(6) Yang, S. F.; Hoffman, N. E. *Annu. Rev. Plant Physiol.* **1984**, *35*, 155-189.
(7) Adams, D. O.; Yang, S. *Trends Biochem. Sci. (Pers. Ed.)* **1981**, *6*, 161-164.
(8) Burg, S. P.; Burg, E. A. *Plant Physiol.* **1967**, *42*, 144-152.
(9) Adams, D. O.; Yang, S. F. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 170-174.
(10) Satoh, S.; Esashi, Y. *Plant Cell Physiol.* **1980**, *21*, 930-949.
(11) Apelbaum, A.; Wang, S.; Burgoon, A.; Baker, J.; Lieberman, M. *Plant Physiol.* **1981**, *67*, 74-79.
(12) Lavee, S.; Martin, G. *Plant Physiol.* **1981**, *67*, 1204-1207.
(13) Hoffman, N. E.; Yang, S. F.; Ichihara, A.; Sakamura, S. *Plant Physiol.* **1982**, *70*, 195-199.
(14) Liu, H.-W.; Auchus, R.; Walsh, C. T. *J. Am. Chem. Soc.* **1984**, *106*, 5335-5348. Walsh, C. T.; Pascal, R. A.; Johnston, M.; Raines, R.; Dikshit, D.; Krantz, A.; Honma, M. *Biochemistry* **1981**, *20*, 7509-7519.
(15) Baldwin, J. E.; Adlington, R. M.; Rawlings, B. J. *Tetrahedron Lett.* **1985**, *26*, 481-484. Baldwin, J. E.; Adlington, R. M.; Rawlings, B. J.; Jones, R. H. *Tetrahedron Lett.* **1985**, *26*, 485-488.
(16) Yang, S. F.; Yu, Y.-B. *Plant Physiol.* **1979**, *64*, 1074-1077.
(17) Pirrung, M. C. *J. Am. Chem. Soc.* **1983**, *105*, 7207-9.
(18) Schollkopf, U.; Harm, R.; Hoppe, D. *Justus Liebigs Ann. Chem.* **1973**, 611-618.

0022-3263/86/1951-2103$01.50/0

flask to a separatory funnel. The organic phase was separated, and the aqueous phase was extracted with 3 × 40 mL of ether. The combined organic phases were washed twice with water to remove Me_2SO and once with brine and then dried over K_2CO_3. Filtration, careful evaporation of the volatiles, and Kugelrohr distillation (180 °C, 18 torr) provided 2.16 g (59%) of the title compound: ^1H NMR ($CDCl_3$) δ 1.15 (1 H, dd, J = 8.5), 1.32 (3 H, t, J = 7), 1.34 (3 H, d, J = 6), 1.78 (2 H, m), 4.23 (2 H, q, J = 7); IR (film) 2980, 2130, 1730, 1395, 1370, 1290, 1255, 1180, 1100, 1060, 1025, 855, 715 cm^{-1}. Anal. Calcd for $C_8H_{11}NO_2$: C, 62.73; H, 7.24; N, 9.14. Found: C, 62.54; H, 7.25; N, 9.18.

(1*R,2*S**)-2-Methyl-1-aminocyclopropanecarboxylic Acid (2).** The isocyano ester above (1.35 g, 8.82 mmol) was dissolved in 10 mL of 5 M KOH and heated at reflux for 2 h. The solution was cooled and neutralized with concentrated HCl. The reaction mixture was loaded onto a 200-g (wet) Dowex 50 (H^+) column and eluted with water until the eluate was neutral. Ammonia (1 M, 300 mL) was used to elute the amino acid, which was obtained after concentration. Recrystallization gave a first crop from 10 mL of 3:1 EtOH/H_2O and a second crop from 5 mL of 3:1 EtOH/H_2O. The combined yield was 333 mg (33%). The mother liquors were retained and used for subsequent preparations: ^1H NMR (D_2O) δ 1.30 (1 H, dd, J = 7.3, 6.1), 1.62 (3 H, d, J = 6.5), 1.87 (1 H, dd, J = 9.7, 6.1), 2.06 (1 H, m); mp 213–214 °C; HRMS calcd for $C_5H_{10}NO_2$ 115.06331, found 115.0629.

(*S*)-2-Bromo-1-propanol. To a 500-mL, round-bottomed flask were added dry tetrahydrofuran (75 mL) and (*S*)-2-bromopropionic acid (92% ee, prepared by the literature procedure,[24] 26.0 g, 0.179 mol). The flask was sealed, purged with N_2, and cooled to 0 °C. Borane/THF (1.0 M, 187 mL) was then added dropwise over 15 min with vigorous gas evolution. The mixture was stirred at 0 °C for 2 h, then a saturated ammonium chloride/5% HCl mixture was added (150 mL/50 mL). This was stirred for 20 min at room temperature. Saturated brine (50 mL) was then added. The tetrahydrofuran layer was separated. The aqueous phase was extracted with methylene chloride (3 × 75 mL). The organic phases were pooled and washed with brine (1 × 50 mL). The organics were dried over anhydrous $MgSO_4$. The solution was filtered, and the solvents were removed, first at atmospheric pressure and then at 100 torr. 2-Bromo-1-propanol was purified by Kugelrohr distillation (70 °C/22 torr, lit. 61 °C/15 torr) to give 15.3 g of product. The spectral data matched those of the racemic series [$[\alpha]_D^{20}$ +4.41 (*c* 1.27, tetrahydrofuran)]. The compound was converted to the MTPA ester (DCC) and found to have 90% ee.

(*S*)-1,2-Dibromopropane. To a solution of (*S*)-2-Bromo-1-propanol (5.0 g, 36.0 mmol) stirring in dry methylene chloride (50 mL) at 0 °C under a nitrogen atmosphere was added methanesulfonyl chloride (3.1 mL, 39.6 mmol) dropwise over 10 min. Triethylamine (5.8 mL, 41.4 mmol) was then added dropwise over 5 min. The mixture was stirred at 0 °C for ~20 min. A saturated sodium bicarbonate solution (70 mL) was then added. The mixture was stirred vigorously for 15 min. The methylene chloride layer was separated. The aqueous phase was extracted with methylene chloride (50 mL). The methylene chloride layers were pooled and dried over anhydrous $MgSO_4$. The organics were filtered and concentrated. Dry tetrahydrofuran (35 mL) and lithium bromide (anhydrous, 3.13 g, 36.0 mmol) were added. The mixture was stirred at reflux for 4 h and cooled. The salts were filtered, and the solvent was removed at atmospheric pressure. Kugelrohr distillation of the residue afforded the dibromide (bp 70 °C/55 torr, lit. 36 °C/10 torr) (5.98 g, 82%). The spectral data matched those of the racemate [$[\alpha]_D^{20}$ −19.43 (neat)].

(1*R*,2*S*)-Methyl 2-Methyl-1-isocyanocyclopropanecarboxylate. The procedure of Schollkopf was followed as described above, using (*R*)-1,2-dibromopropane, to afford 1.2 g of the title compound in 27% distilled yield. Spectral data were identical with those of the racemic series.

(1*R*,2*S*)-2-Methyl-1-aminocyclopropanecarboxylic Acid. To methanol (10 mL) stirring at 0 °C was added acetyl chloride (0.77 mL, 10.8 mmol). This solution was stirred for 10 min. The (1*R*,2*S*)-methyl 2-methyl-1-isocyanocyclopropanecarboxylate (1.00 g, 7.19 mmol) was then added dropwise over 2 min. The mixture was stirred for 24 h at room temperature. It was concentrated in vacuo to give an off-white powder. To this was added methanol (10 mL) and potassium hydroxide (1.04 g, 18.5 mmol). This was stirred at room temperature for 24 h; it was then neutralized and loaded onto a 50-g Dowex 50 (H^+) column. This was eluted as described above to afford the title compound (510 mg, 65%). Recrystallization from ethanol/water gave homogeneous material whose spectral data were the same as described for the racemate [$[\alpha]_D^{25}$ +75.5 (*c* 0.24, H_2O)].

Treatment of a sample of this compound suspended in methanol with enough $SOCl_2$ to form a 2 M HCl solution gave the methyl ester after 4 h at 40 °C and evaporation of the volatiles. Methylene chloride was added, followed by 3 equiv of (−)-MTPA, 3 equiv of DCC, and 1.1 equiv of DMAP. After stirring at room temperature for 18 h, 10 equiv of lactic acid were added. Dilution with 10 vol of ether precipitated salts which were removed by filtration. The filtrate was washed with 5% HCl and 3 times with saturated $NaHCO_3$. Drying and filtration through a plug of silica gel gave a solution which was evaporated to give the MTPA-amide methyl ester. Examination of this substance by ^{19}F NMR showed signals at 8.73 ppm (1*R*,2*S*) and 8.66 ppm (1*S*,2*R*) relative to external TFA. The enantiomeric excess was found to be 91%.

(1*S*,2*R*)-2-Methyl-1-aminocyclopropanecarboxylic Acid. This compound was prepared as described for the 1*R*,2*S* enantiomer above to afford the title compound in 20% overall yield for the alkylation and two-step hydrolysis. Recrystallization from ethanol/water gave homogeneous material [$[\alpha]_D^{25}$ −67.4 (*c* 0.27, H_2O)]. Conversion to the methyl ester/MTPA amide and NMR analysis as above demonstrated the enantiomeric excess to be 82%.

(1*R,2*S**)-Methyl 2-Ethyl-1-isocyanocyclopropanecarboxylate.** The procedure of Schollkopf was followed as described above, using 1,2-dibromobutane, to give 1.3 g of the title compound in a 29% distilled yield (bp 113 °C/14 torr). The compound is a 9/1 mixture of diastereomers: IR (film) 2140, 1735, 1435, 1310, 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.11 (3 H, t, J = 7), 1.24 (1 H, m), 1.64 (2 H, m), 1.78 (2 H, m), 3.83 (3 H, s).

(1*R,2*S**)-1-Amino-2-ethylcyclopropanecarboxylic Acid.** The two-step hydrolysis described above was employed. Thus, to dry methanol (5.0 mL) stirring at 0 °C, acetyl chloride (0.46 mL, 6.5 mmol) was added dropwise. This solution was stirred for 10 min. The (1*R**,2*S**)-Methyl 2-ethyl-1-isocyanocyclopropane carboxylate (0.500 g, 3.22 mmol) was then added dropwise over 2 min. The mixture was stirred for 24 h at room temperature. It was concentrated in vacuo to give an off-white powder. To this was added methanol (5.0 mL) and potassium hydroxide (0.54 g, 3.0 equiv). This mixture was stirred at room temperature for 24 h. The mixture was neutralized with 5% HCl and loaded onto a 25-g Dowex 50 (H^+) column. It was eluted as described above to give the title compound (319 mg, 78%). Recrystallization from ethanol/water gave homogeneous material whose spectral data matched those described by Baldwin.[15]

Results

The preparation of (1*R*,2*S*)-2-methyl-1-aminocyclopropanecarboxylic acid (MeACC) was achieved by applying Schollkopf's procedure to 1,2-dibromopropane and ethyl isocyanoacetate. Compared to many other protected glycine dianion equivalents examined for ACC synthesis (benzylidene,[14,19] BOC, and stabase glycine), ethyl isocyanoacetate has in our hands proved far superior.[17,20–22] The products are easily obtained from a variety of dibromides after bulb-to-bulb distillation with little contamination from starting material. With 1,2-dibromoalkanes, the alkylation not only proceeds effectively but it does so with a high degree of diastereoselection for the anti relationship of the alkyl and carboxyl groups. NMR analysis of the products of cycloalkylation with methyl-, ethyl- (see Experimental Section), and cyclopropyl[20]-substituted dibromides shows greater than 85% diastereo-

(19) Adlington, R.; Rawlings, B.; Baldwin, J. *J. Chem. Soc., Chem. Commun.* **1983**, 290–292.
(20) Pirrung, M. C.; McGeehan, G. M. *Angew. Chem.* **1985**, *97*, 1074.
(21) O'Donnell, M.; Bruder, W. A.; Ekrich, T. M.; Schullenberger, D. F.; Staten, G. S. *Synthesis* **1984**, 127–128.
(22) Ramalingam, K.; Kalvin, D.; Woodard, R. W. *J. Labeled Compd. Radiopharm.* **1984**, *21*, 833–841.

meric purity. These are carried on by hydrolysis to the amino acids which are stereochemically homogeneous after recrystallization. The stereochemical assignment rests on comparison to published spectral data for both diastereomers.[14,15] The stereochemical outcome may be rationalized by analogy to the results of Stork in epoxy–nitrile cyclizations.[23]

In the optically active series, a source for dibromopropane in either enantiomeric form is alanine. Following literature procedures,[24] L- or D-alanine was diazotized to form 2-bromopropionic acid, reduced (BH_3·THF), and brominated (MsCl, Et_3N; LiBr, THF). The use of D-alanine, which is inexpensive, was preferrable to the Seebach procedure in which L-alanine is used, and the chiral center is inverted via the epoxide in order to obtain the antipode. The enantiomeric purity was assessed at the bromopropanol stage using the MTPA ester[25] and was found to be greater than 90% ee. Subjection of the dibromides to the alkylation/hydrolysis protocol above gave the optically-active (1*R*,2*S*)- or (1*S*,2*R*)-2-methyl-1-aminocyclopropanecarboxylic acids. They show opposite rotations (1*R*,2*S* – $[\alpha]_D^{25}$ +75.5°, 1*S*,2*R* – $[\alpha]_D^{25}$ –68.2°), and their enantiomeric purity was assessed by conversion (MeOH, $SOCl_2$; MTPAOH, DCC) to the methyl ester MTPA-amide. Examination of their ^{19}F NMR spectra allowed a determination of optical purity as follows: 1*R*,2*S* – 91% ee, 1*S*,2*R* – 82% ee. These results provide independent confirmation of Baldwin's assignment of absolute configuration.[15]

A plant tissue is required for the evaluation of MeACC in ethylene biosynthesis, since no cell-free system which conducts authentic ethylene biosynthesis is known. A modification of Yang's system, mung bean hypocotyl segments, is very useful.[16] In the absence of externally applied auxin, this tissue is not capable of ACC biosynthesis. Since the ethylene-forming enzyme (EFE) is constitutive, mung bean segments may be treated as a "black box" which represents the EFE. Application of substrates or inhibitors leads to reactions which can be characterized by standard kinetic methods.

Some hazards obviously exist in using a tissue to represent an enzyme. The effects observed might be due not to the EFE but to enzymes involved in uptake, transport, or modification[26] of analogues which may differ appreciably from substrate. There is evidence, however, that uptake and transport are *not* limiting in the processing of analogues.[27] With these *caveats*, model systems still provide a useful means to study the EFE, and since an ultimate goal for modifying plant physiological processes would be to develop compounds to inhibit it in vivo, whole tissue systems may provide some advantages over isolated enzymes.

Racemic MeACC was used for the competition studies. Based on results in the ethyl series,[13] it was expected that only one antipode of this diastereomerically pure analogue would be processed by the EFE. Study of the rate of processing of both of the optically active compounds and the racemate permitted a determination of the relative rate for the pure enantiomers. As Figure 1 shows, the rate of processing of the 1*R*,2*S* stereoisomer is greatest. The intrinsic rates for optically pure enantiomers were determined from these raw rates by least-squares analysis and

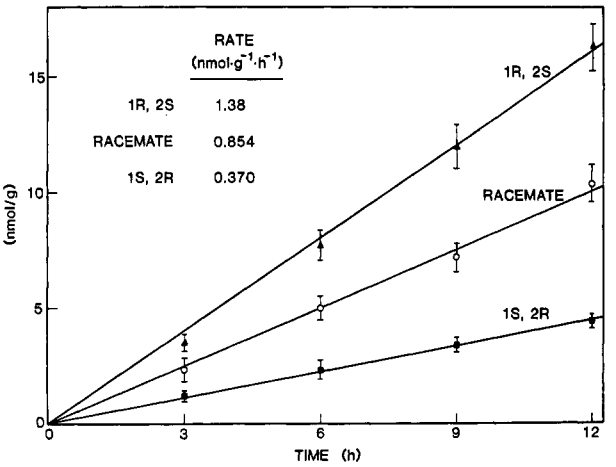


Figure 1. Processing of stereoisomers of MeACC by mung bean hypocotyl segments. (A) 1*R*,2*S*; (B) racemate; (C) 1*S*,2*R*.

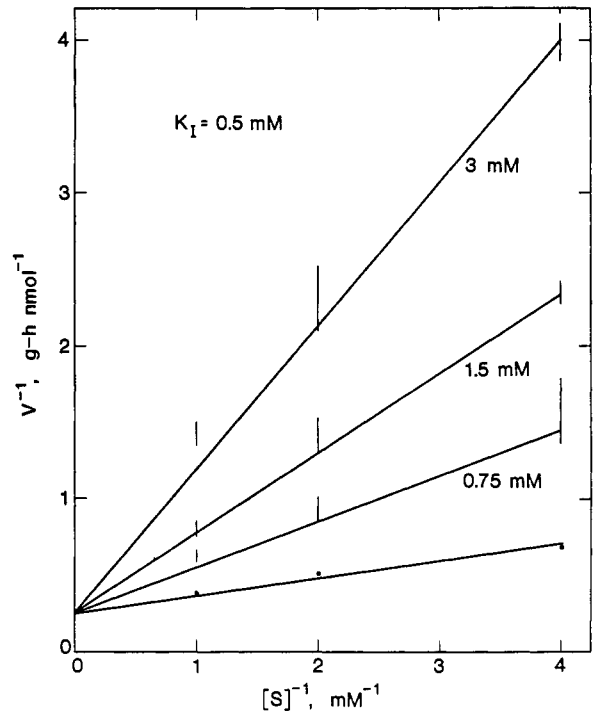


Figure 2. Lineweaver–Burk analysis of the inhibition of ethylene biosynthesis from ACC in mung bean hypocotyl segments by MeACC. Lines represent least-squares fits to data from a single experimental run using one mung bean group. Bars represent range of values over multiple runs.

were found to be 9:1. This result is consistent with Yang's and provides experimental justification for using the more abundant racemic amino acid and correcting the kinetic parameters for the portion of the compound which will be processed. As expected, MeACC is processed to propene by mung bean hypocotyl segments, showing saturation kinetics with an apparent K_m of 0.5 mM. This is simply a concentration in solution which leads to half-maximal activity for propylene production. For comparison, the kinetics of processing of ACC under these conditions were also determined; within experimental error K_m is the same, 0.5 mM. When applied to different samples of tissue in identical concentration, MeACC is processed to propene at 80% of the rate at which ACC is processed to ethylene. This rate difference derives exclusively from a K_{cat} effect.

While Yang reports that allocoronamic acid (1) is a substrate for the ethylene-forming enzyme, it is critical to show that MeACC is a true substrate in the sense that it reacts competitively with ACC at the same site. The Li-

(23) Stork, G.; Cohen, J. *J. Am. Chem. Soc.* **1974**, *96*, 5270–5272.
(24) Amarego, W.; Milloy, B.; Pendergast, W. *J. Chem. Soc., Perkin Trans. 1* **1976**, 2229–2237. Navera, C.; Tos, M.; Seebach, D. *Helv. Chem. Acta* **1984**, *67*, 289–300.
(25) Dale, J.; Dull, J.; Mosher, H. *J. Org. Chem.* **1969**, *34*, 2543.
(26) Liu, Y.; Hoffman, N. E.; Yang, S. F. *Planta* **1983**, *158*, 437–441.
(27) Venis, M. A. *Planta* **1984**, *162*, 85–88.

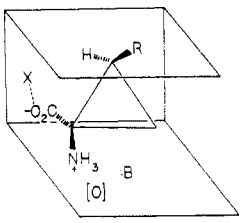


Figure 3. Active-site cartoon of the ethylene-forming enzyme.

neweaver–Burk analysis in Figure 2, which yields identical values of K_I and K_m, 0.5 mM, provides necessary but not sufficient proof that this is the case.

Discussion

This study establishes MeACC as a substrate and inhibitor of the ethylene-forming enzyme. The characteristics of its processing by the EFE are surprising. A priori, its 20% slower turnover might have been attributed to steric interactions which cause looser binding. No change in K_m and a smaller K_{cat} suggests that steric factors, while unimportant in binding, do become important in the transition state.

MeACC is the strongest competitive inhibitor of the EFE yet described. Its preparation from optically pure precursors of unambiguous absolute configuration provides a useful route to this chiral analogue as well as firmly establishing the chirality required of analogues by the active center for ethylene production. The synthetic methodology used provides a general route to alkylated ACC derivatives in just two steps from 1,2-dibromides.

The fact that MeACC is a better substrate than the corresponding ethyl compound, along with our other previous results,[17] allows us to further refine the active-site picture of the EFE as shown in Figure 3. These additions include a "roof" which interacts with alkyl groups at the 2-position; a binding group X which interacts with the carboxyl group, since neither alkyl groups[10-12] or hydroxymethyl (Pirrung, M. C., unpublished) can substitute at this position; bases in position to remove protons from nitrogen as the oxidation proceeds; and an oxidant positioned adjacent to nitrogen. The nature of this oxidant is currently unknown. Our previous model work has shown that one-electron oxidation can lead to ethylene production[17] but also indicates that a hydrogen atom-abstracting oxidant could accomplish the desired goal.[28] Baldwin has shown that high-valent metal oxo derivatives may also be competent.[29] What this picture does not adequately explain is how this reputedly "tight" active site allows α-aminoisobutyric acid to enter,[30] or how free-radical traps like propyl gallate intercept the intermediates in ethylene formation.

MeACC may have another use in ethylene biosynthesis studies. It has recently been established[31,32] that cyanide is produced from C1–N1 of ACC during ethylene biosynthesis. It has been suggested[32] that of the physiological changes associated with ethylene biosynthesis, the shift to a cyanide-insensitive electron transport system may be connected with cyanide production. Since MeACC is converted to propene, which has ca. 60 times less physiological activity than ethylene,[8] yet will also produce cyanide, a comparison of the physiological responses to ACC and MeACC will allow the effects of each substance to be sorted out. Such studies are currently underway.

Acknowledgment. Advice from Professor Mosher concerning the determination of optical purity is appreciated. Financial support from FMC Corp. and the U. S.–Israel Binational Agricultural Research and Development Fund, Grant No. I-643-83, is gratefully acknowledged. NMR spectra were obtained on a Nicolet NMC-300 spectrometer purchased with funds provided by the National Science Foundation (CHE-8109064).

Note Added in Proof: Baldwin has recently reported the results of processing of the alkyl deuterated ACC's: Baldwin, J. E.; Adlington, R. M.; Lajoie, G. A.; Rawlings, B. J. *J. Chem. Soc., Chem. Commun.* **1985**, 1496–1498.

(28) Pirrung, M. C. *Biochemistry* **1986**, *25*, 114–119.
(29) Baldwin, J. E.; Jackson, D. E.; Adlington, R. M.; Rawlings, B. J. *J. Chem. Soc., Chem. Commun.* **1985**, 206–207.
(30) Yang, S. F.; Liu, Y.; Su, L. *Planta* **1984**, *161*, 439–443.
(31) Peiser, G. D.; Wang, T.-T.; Hoffman, N. E.; Yang, S. F.; Liu, H.-W.; Walsh, C. T. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 3059–3063.
(32) Pirrung, M. C. *Bioorg.* Chem. **1985**, *13*, 219–226.

Analysis of the Factors Contributing to the Large Changes in the Product Distributions Observed in Radical-Chain Addition and Cycloaddition Reactions of Ethylallene and Ethylallene-*3,3*-d_2

Daniel J. Pasto,* Steven E. Warren, and Thomas Weyenberg

Department of Chemistry, University of Notre Dame, Notre Dame, Indiana 46556

Received November 29, 1985

The radical-chain addition of benzenethiol to ethylallene (ETA) and to a 1:1 mixture of ETA and 3,3-dideuterioethylallene (ETA-d_2) produces substantially different product distributions (Scheme I). Much larger differences in product (and d_2) distributions are observed in the cycloaddition reactions with 1,1-dichloro-2,2-dichloroethene (1122) and *N*-phenylmaleimide (NPMI) (Schemes II and III). These changes in product distributions are attributed to four cooperative effects: a difference in the regioselectivity for reaction between the (*E*)- and (*Z*)-ethyl-substituted allyl radicals which is steric in nature favoring reaction at the ethyl-substituted end of the allyl radical in the (*Z*)-isomer and three isotope effects. Deuterium substitution is shown to favor formation of the (*Z*)-alkyl-substituted allyl radical (a steric isotope effect) and also to favor atom transfer and ring closure at the ethyl-substituted end of the allyl radical in the intermediates. The ring-closure reaction is dominated by a rotational isotope effect.

During the past decade efforts in the author's laboratories have been directed toward gaining a thorough understanding of the mechanistic details of addition and cycloaddition reactions of substituted allenes. The results

0022-3263/86/1951-2106$01.50/0