Ethylene Biosynthesis. Aminocyclopropenecarboxylic Acid

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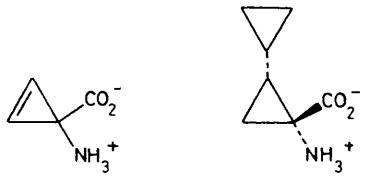
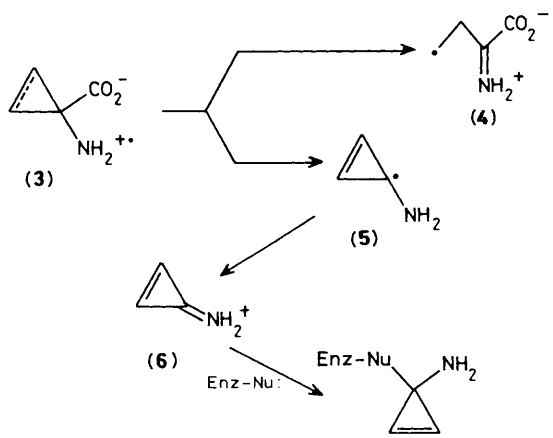
A new time-dependent inhibitor of ethylene biosynthesis, 1-aminocyclopropenecarboxylic acid, has been shown to be an extremely poor substrate for acetylene production as well as a preventative of senescence; the inhibiting species for this cyclopropene analogue of aminocyclopropanecarboxylic acid (ACC), is postulated to be an aromatic cyclopropene imine.

The design of specific modulators of ethylene biosynthesis is an important goal. Such materials have the potential to serve as tools in plant breeding efforts[1] or as preservatives of agricultural products by preventing senescence.[2] One of the compounds most useful in preventing ethylene production is aminoethoxyvinylglycine (AVG),[3] a fermentation product which inhibits the pyridoxal enzyme responsible for the biosynthesis of aminocyclopropanecarboxylic acid (ACC), the ethylene precursor.[4] Because the ethylene-forming enzyme has little in common with other metabolic pathways, it is more attractive than ACC-synthase as a target for inhibitor design. Only limited success has been reported towards this goal, however.[5] An intriguing possibility for development of mechanism-based ($k_{cat.}$) inhibitors[6] involves the diversion of ACC-radical cation (**3**), which has been proposed as a key intermediate in ethylene biosynthesis,[7] from the normal ring opening to decarboxylation (Scheme 1). This might be accomplished, for example, by incorporation of a double bond into the three-membered ring, leading to a product (**6**) which benefits from aromatic stabilization but which also is highly reactive. This report concerns the processing of such an ACC analogue, which is indeed a $k_{cat.}$ inhibitor of the ethylene-forming enzyme and an inhibitor of senescence.



(**1**) (**2**)



Scheme 1

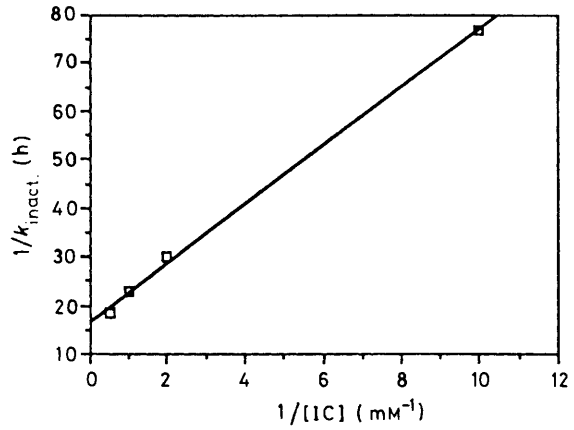


Figure 1. Double reciprocal plot of the observed rate constant for inactivation (h, *y*-axis) *vs.* inhibitor concentration (mM^{-1}, *x*-axis).

1-Aminocyclopropenecarboxylic acid (**1**) is available *via* a published route[8] from dimethyl diazomalonate and bis-(trimethylsilyl)acetylene. In side-by-side comparison with ACC at 0.5 mM in apple tissue, (**1**) is converted to acetylene at 1/500th the rate ACC is converted to ethylene. (No acetylene is detectable until 10 h.) This difference cannot be due to the inability of (**1**) to access the active site, since its IC_{50} (determined in mung bean hypocotyl segments[9]) is 0.3 ± 0.05 mM, compared to the 0.5 mM IC_{50} found for methyl-ACC[11] and the 0.5 mM concentration of ACC which promotes half-maximal ethylene production in this sytem.

Chemical models have proven extremely useful in making mechanistic inferences concerning the processing of ACC and its analogues in plants. Two of interest include the electrochemical oxidation in a non-aqueous system[7] and a peroxide–metal ion model.[10] Both proceed with scrambling of stereochemistry, and free-radical mechanisms have been proposed. On treatment with the Mn^{2+}/H_2O_2/salicylaldehyde system,[10a] (**1**) is converted to acetylene and cyanide at a rate of 60 nmol/h. By comparison, ACC is processed at 160 nmol/h. Analogous to the *in vivo* results, electrochemical oxidation[7] of (**1**) produces barely detectable quantities of acetylene ($k_{rel.}$ = 1/300).

This situation is reminiscent of that pertaining to cyclopropyl-ACC (**2**),[11] which is also a good inhibitor but poor substrate. Since (**1**) possesses the functionality necessary for processing, its low activity could suggest that some enzyme-bound intermediate is being diverted. An assay for time-dependent loss of ethylene-forming enzyme induced by (**1**) in mung bean hypocotyl segments was therefore conducted. Replot of the slopes for the kinetics of activity loss (Figure 1) demonstrates saturation and allows the estimation of $k_{inact.}$ = 1.9×10^{-5} s^{-1} for this $k_{cat.}$ inhibitor. For comparison, $k_{inact.}$ = 1.7×10^{-5} s^{-1} for (**2**). The low amounts of gaseous product formed from both (**1**) and (**2**), despite their being good competitive inhibitors, suggest a low partition ratio (inactivation/turnover).

Given its ability to inhibit ethylene production, the physiological properties of (**1**) were studied in a carnation anti-senescence assay (Figure 2).[12] Data on (**2**) are also included.[13] A demonstrable effect on flower quality can be ascribed to both compounds, which is comparable to that of AVG at submillimolar concentrations.

An approach to the modification of the physiological activities of plants by inactivation of the ethylene-forming enzyme is hereby demonstrated. It may be postulated that the

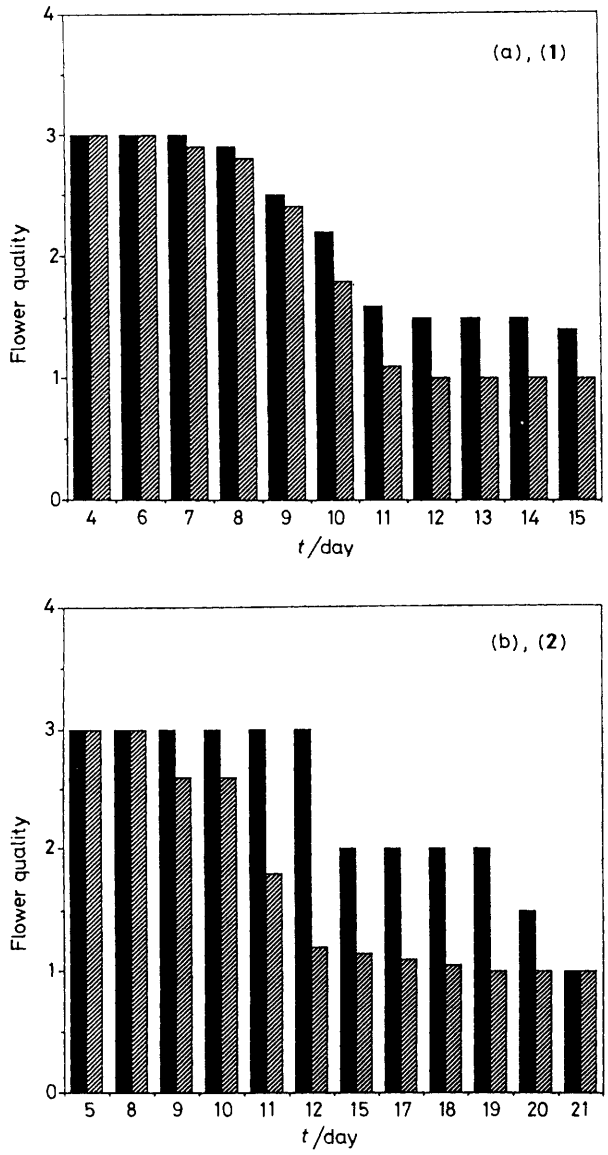


Figure 2. Anti-senescence assay of (a) (**1**) and (b) (**2**) in the system described by Eisinger.[12] The *y*-axis represents flower quality (subjective). Inhibitors were provided at 0.5 mM (**1**) and 5 mM (**2**). The hatched bars represent controls.

key to the effect of (**1**) is its ability to form the stabilized cyclopropenone imine (**6**), which can be subject to nucleophilic attack by an enzymic residue. While cyclopropene imines[14] are known, unsubstituted versions of the imine and immonium ion have been studied only by theoretical methods.[15] However, the ion responsible for the base peak (*m/z* 54) in the mass spectrum of ACC has been tentatively assigned the structure (**6**).[16] An intermediate in the path to (**6**) *in vivo* or in the gas phase is presumably radical (**5**), which is expected to adopt a σ structure.[17] An additional possibility is that (**1**) is a slow, tight-binding inhibitor. Its participation as a substrate makes this less likely, however. The determination of the course of inhibition and the development of routes to other cyclopropane-based irreversible inactivators will constitute future goals.

Support was provided by the National Science Foundation (CHE 84-51324) and the Schweizerische Nationalfonds (Postdoctoral fellowship to U. P. T.). Rhone-Poulenc Ag Company

is thanked for a sample of 1-aminocylopropenecarboxylic acid. Prof. W. Eisinger is thanked for helpful discussions and the anti-senescence assay of (**2**). M. C. P. is a Research Fellow of the Alfred P. Sloan Foundation, 1985—1989, and Presidential Young Investigator, 1985—1990.

Received, 7th February 1989; Com. 9/00573K

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