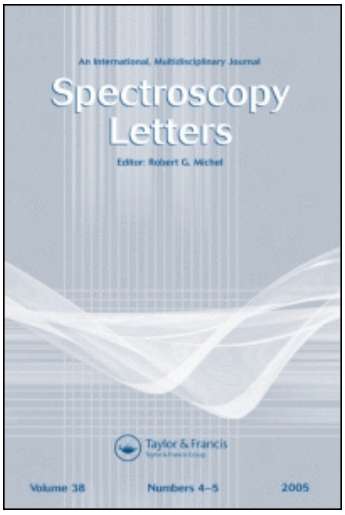

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THE PROTON NMR ASSIGNMENT OF 1-AMINOCYCLO-PROPANE-1-CARBOXYLIC ACID.

Key words : ^{1}H-nmr, Shift reagents, Deuterium labeling, 1- aminocyclo-propane-1-carboxylic acid

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Abstract: The two sets of diastereomeric hydrogen atoms *cis* and *trans* to the carboxylate of 1-aminocyclopropane-1-carboxylic acid (ACC) were differentiated by a ^{1}H-nmr study. The measurement of the ^{1}H-nmr spectrum of ACC at pH 3.8 during the successive addition of non-chiral lanthanide reagents such as $Eu(NO_3)_3$, $Pr(NO_3)_3$ or $Gd(NO_3)_3$ demonstrated that the hydrogen atoms *cis* to the carboxylate function of ACC resonate at δ=1.42 and that the hydrogen atoms *trans* to the carboxylate function of ACC resonate at δ=1.20. The mono-substituted cyclopropanes, cyclo-propanecarboxylic acid (CPC) and cyclopropyl-amine (CPA) were used in complementary lanthanide-reagent shift titrations along with the back titration of an ACC-Eu^{+++} complex with DCl to further substantiate the assignment. This assignment allows for the non-destructive, nonisotopic diluting analysis of various biosynthetically derived deuterated ACC's formed from the corresponding deuterated *S*-adenosyl-L-methionine.

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INTRODUCTION

Our studies (1) on the mechanism of the biosynthesis of 1-amino-cyclopropane-1-carboxylic acid (ACC) from *S*-adenosyl-L-methionine (SAM) by 1-aminocyclopropane-1-carboxylic acid synthase (2-4) and our interest in the synthesis of regio- and stereo-specifically deuterium-labeled 1-aminocyclo-propane-1-carboxylic acid (5) for the study of the mechanism of the biosynthesis of ethylene by plants has made it necessary to distinguish between the diastereotopic (*cis* and *trans*) hydrogens of ACC (Fig. 1).

The ^{1}H-nmr spectrum of ACC in D_2O shows two multiplets centered at δ=1.20 (2H) and δ=1.42 (2H). The exact assignment of which signal represents the hydrogen atoms *cis* to the amino group and which represents those *trans* is the key to the analysis of the stereochemical outcome of the reaction catalyzed by ACC synthase. The chemical shift of each set of hydrogen atoms (*cis* or *trans* to the amino or carboxylate function) depends on several factors, one of which is the through-space effect of the zwitterion at C-1, the predominant form of ACC at pH's = 3-8. The assumption that a carboxylate anion provides a net shielding effect to *cis* hydrogens in a planar ring (6) would lead one to predict that the upfield multiplet belongs to the hydrogens *cis* to the carboxylate group. Another report (7) suggests that the carboxylate function generally causes a deshielding of the hydrogens *cis* to the carboxylate group. Some typical δ-values for mono-substituted cyclopropanes are given in Table 1 (8). As can be seen from the δ-values of the substituted cyclopropanes, the hydrogen atoms *cis* to a carbonyl function resonate downfield of the hydrogen atoms *trans* to a

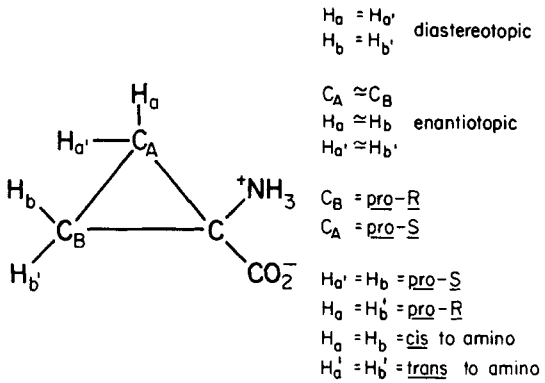

Figure 1. Stereochemical Relationships of the hydrogen atoms and the carbon atoms in ACC.

TABLE 1.

Typical δ-values of *cis* - and *trans* - hydrogens in Monosubstituted Cyclopropanes (8).

Substituent	δ-value of ^{1}H *cis* [a]	δ-value of ^{1}H *trans* [a,b]
$-CH_2OH$	0.1747	0.4600
-CHO	1.0262	0.9872
$-COCH_3$	0.9305	0.7665
$-CO_2H$	1.0453	0.8828
$-NH_2$	0.2220	0.3276

[a] Chemical shifts ppm downfield from TMS.

[b] Exclusive of geminal proton.

carbonyl function, whereas the hydrogen atoms *cis* to an amino function resonate upfield of the hydrogen atoms *trans* to an amino function.

Based on these typical values, we have tentatively assigned the downfield signal (δ=1.42) to be the hydrogen atoms *cis* to the carboxylate function. There are several methods one may use in order to verify this assignment: 1. Conversion of the carboxylate to derivatives of lower oxidation 2. Use of model compounds such as coronamic acid (1*S*,2*S*-2-ethyl-1-aminocyclopropane-1-carboxylic acid) in which the X-ray data and ^{1}H-nmr data are available (9) 3. Use of neutron diffraction methods (10) 4. Use of lanthanide shift reagents. The limited amount of compounds and our desire to analyze in a non-destructive, non-derivatizing and non-isotopic diluting technique suggested the latter procedure of using a shift reagent.

Reuben (11), Staniforth et al. (12) and Miyazawa et al. (13) have reported the use of non-chiral aqueous lanthanide reagents to selectively shift the ^{1}H-nmr signal of the various hydrogen atoms in α-hydroxy-carboxylates, hydroxy- or amino-acids, and amino acids respectively. Recently two reviews have appeared describing the use of aqueous lanthanide reagents in the elucidation of the ^{1}H-nmr assignments (14,15).

We report herein the assignment of the ^{1}H-nmr of ACC based on studies with $Eu(NO_3)_3$, $Pr(NO_3)_3$ and $Gd(NO_3)_3$ in D_2O at pH=3.8.

EXPERIMENTAL SECTION

The cyclopropanecarboxylic acid, cyclopropylamine, the 37 wt. % deuterium chloride and deuterium oxide (100% ^{2}H) were purchased from Aldrich Chemical Company. The cyclopropane compounds were

distilled prior to use. The $Eu(NO_3)_3 \cdot 6H_2O$, $Pr(NO_3)_3 \cdot 6H_2O$ and $Gd(NO_3)_3 \cdot 9H_2O$ were purchased from Morton Thiokol, Inc. and the waters of hydration exchanged with D_2O via repeated freeze-drying. Stock solutions of the lanthanide nitrates ($Ln(NO_3)_3$) were made up to 1.5 M so that the sequential additions of the lanthanide solution to the test compounds could be made without any significant change in the concentration of the test compound. The 360.13-MHz or 270.13-MHz ^1H-nmr spectra were obtained with a Bruker WM-360 and an IBM WP-270-SY FT NMR spectrometer with a pulse width of 2 and 3 μs, respectively with 16 K data points for a spectral width of 6260 Hz and with an exponential line broadening of 0 Hz. The 360 MHz or 270 MHz ^1H-nmr spectra of a solution of ACC-d_2 (0.15 M in D_2O, pH = 3.8) were obtained after each sequential addition of a 1.5 M solution of $Ln(NO_3)_3$ up to a final molar ratio, $\rho(Ln^{+++}/ACC)$, of 1.0 at 26.5°C. The pH was adjusted with DCl and measured directly in the nmr tube using a pH electrode 3.5 mm o.d. (extra long) from Aldrich Chemical Co. with Hg/Hg_2Cl_2 as reference. The values reported for pH's represent the actual pH meter reading and not mathematical corrections. The chemical shift of each doublet is expressed in ppm referenced to dioxane, which was present at a concentration of 0.6%.

The D,L-1-amino-[2,2-$^2H_2$]cyclopropane-1-carboxylic acid was synthe-sized by the method of Ramalingam *et. al.* (5).

RESULTS AND DISCUSSION

In order to simplify the ^1H-nmr spectra of ACC, we synthesized D,L-[2,2-$^2H_2$]ACC (5) which has, on the same carbon atom, one

hydrogen atom *cis* and one hydrogen atom *trans* to the carboxylate function and conversely on the other carbon atom one deuterium atom *cis* and one deuterium atom *trans* to the carboxylate function (i.e. either H_a and $H_{a'}$ or H_b and $H_{b'}$ are deuterium). This simplifies the ^{1}H-nmr spectrum of ACC from an AA'BB' spin system into two doublets centered at δ=1.20 and δ=1.42 (J = 5.7 Hz).

The D,L-[2,2-$^2H_2$]ACC was dissolved in D_2O and the pH adjusted to 3.8 with DCl and the ^{1}H-nmr spectra recorded after sequential additions of lanthanide shift reagent. A plot of the mole ratio, $\rho(Eu^{+++}/ACC)$, versus the shift of the two doublets gave intersecting lines, indicating that a crossover of the doublets has occurred. This can be seen in Figure 2 where the ^{1}H-nmr spectra are plotted, the downfield doublet (δ=1.42) having shifted upfield faster than the upfield doublet (δ=1.20) had shifted upfield (16). At the crossover point (ρ 0.3), the two merging doublets coalesce into a singlet, indicating total magnetic equivalence of the two hydrogen atoms. Therefore, it appears that in the ^{1}H-nmr spectrum (with no Eu^{+++} added) that the doublet at δ=1.42 represents the hydrogen atom *cis* to the carboxylate if indeed Eu^{+++} complexation occurs with the carboxylate and not with the protonated amino function as would be expected at pH = 3.8. When the corresponding experiment was performed with $Pr(NO_3)_3$, the doublets shifted downfield as expected with the doublet at δ=1.42 moving downfield faster than the doublet at δ=1.20. In order to test our hypothesis that the lanthanide-induced shifts are due to complexation of the lanthanide with the carboxylate function and not with the protonated amine function, comparable experiments were performed on non-deuterated cyclopropanecarboxylic acid (CPC) and cyclopropylamine

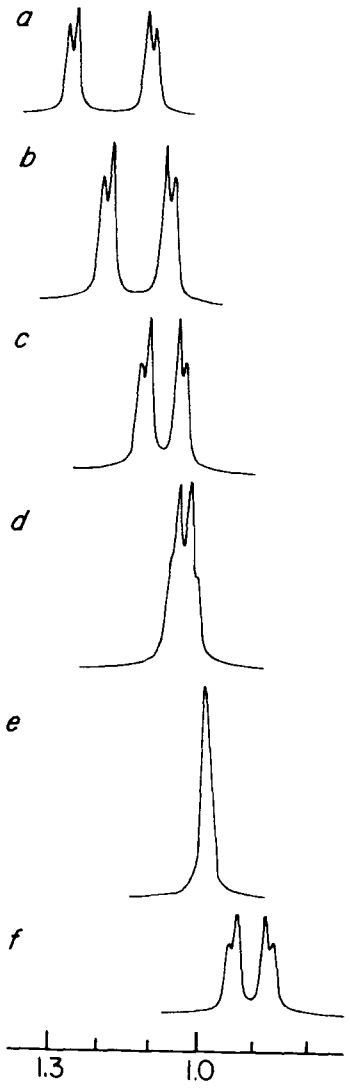

Figure 2. The 360 MHz ^{1}H-nmr spectra of ACC-d_2 (0.149 **M**) in the presence of increasing concentrations of $Eu(NO_3)_3$ (1.5 **M**). The molar ratio [ρ(Eu^{+++}/ACC)] is a. 0, b. 0.07, c. 0.14, d. 0.21, e. 0.27, d. 0.40.

(CPA), in which the assignment of each hydrogen atom has been made (8). The results with CPC, although less dramatic than in the ACC case, demonstrate that with Eu^{+++} at pH = 3.8 in D_2O the hydrogen atoms *cis* to the carboxylate are shifted upfield faster than the other hydrogen atoms, suggesting that at pH=3.8 the Eu^{+++} does complex with the carboxylate function. Results from the Pr^{+++} shift titration, a downfield shift in the resonance of the hydrogen atoms cis to the carboxylate function, also suggest that the carboxylate is the site of complexation. The titration of CPA with either Eu^{+++} or Pr^{+++} in D_2O at pH = 3.8 gave no shift in either of the ^1H-nmr signals due to lack of complexation since the amino function is protonated. To provide further evidence that the lanthanides were complexing with only the carboxylate function of ACC, a complex of Eu^{+++} and ACC-d_2 (ρ=0.6) was prepared at pH = 3.8 in an nmr tube and the ^1H-nmr spectra were recorded with successive additions of DCl. A downfield shift of the doublets with the doublet of lower chemical shift recrossing the doublet of higher chemical shift was observed. We believe that the added D^+ is displacing the Eu^{+++} and effectively "back-titrating" the carboxylate function. Based on the results from the titration of CPC and CPA and the results from the titration of the Eu^{+++} : ACC-d_2 complex with DCl, we believe the site of complexation (or pseudo-contact) of the lanthanide at pH=3.8 in D_2O is the carboxylate function.

A final titration with $Gd(NO_3)_3$, a lanthanide reagent known to effect line broadening of ^1H-nmr signals of hydrogen atoms in a manner proportional to the distance from the Gd^{+++} without any significant shifting of peaks (17), was performed by successive additions to an aqueous solution of ACC-d_2 at pH=3.8. The results shown in Figure 3, in

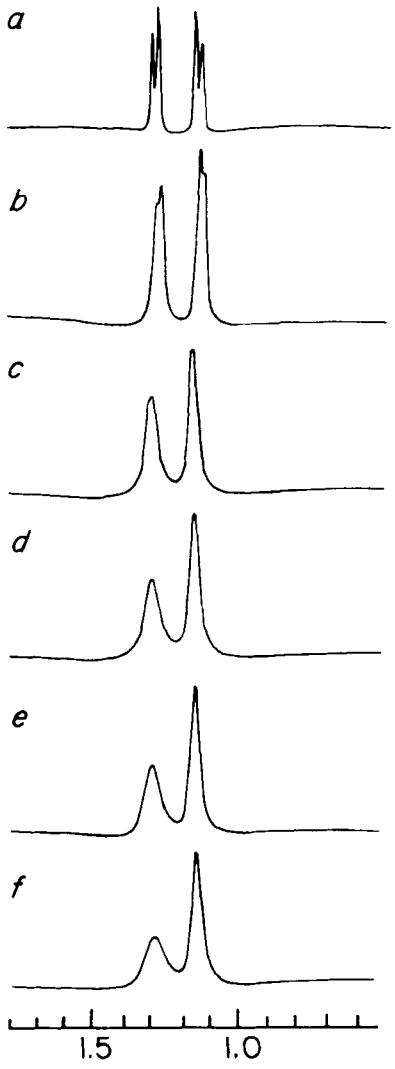

Figure 3. The 270 MHz ^{1}H-nmr spectra of ACC-d_2 (0.15 **M**) in the presence of increasing concentrations of $Gd(NO_3)_3$ (1.0 **M**). The molar ratio [$\rho(Gd^{+++}/ACC)$] is a. 0, b. 0.11, c. 0.22, d. 0.33, e. 0.44, d. 0.66.

which only the signal at δ=1.42 (the hydrogen atoms *cis* to the carboxylate function are closer to the Gd^{+++} than the *trans* hydrogen atoms and therefore should be more broadened) is broadened, confirm that the doublet at δ=1.42 in the ^{1}H-nmr spectrum of ACC-d_2 is due to the hydrogen atoms *cis* to the carboxylate function.

In conclusion, we have shown by various titration experiments with non-chiral lanthanide reagents that the hydrogen atoms *cis* to the carboxylate function resonate at δ=1.42 and that the resonance signal at δ=1.20 is due to the hydrogen atoms *trans* to the carboxylate function. This assignment allows for the non-destructive, nonisotopic diluting analysis of various biosynthetically derived deuterated ACC's formed from the corresponding deuterated *S*- adenosylmethionines.

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