very closely related to the lysine-derived pomegranate bark alkaloid, pseudopelletierine (**4**)[15]. Nevertheless, the substitution pattern in adaline has led to the suggestion that this alkaloid is actually a polyketide[12,13]. In any event, it seems likely that adaline and euphococcinine arise via common biosynthetic pathways.

Deterrency to ants has been demonstrated for one other coccinellid alkaloid (coccinelline)[16]. The possibility that these alkaloids generally, including euphococcinine, are deterrent also to non-arthropodan enemies remains open. Potentially, at least, these compounds could fulfill antivertebrate, antihelminthic, and antimicrobial roles. The laboratory finding[16] that quail prefer alkaloid-free over alkaloid-laden coccinellids is intriguing in this regard.

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Biosynthesis of 1-aminocyclopropanecarboxylic acid: Steric course of the reaction at the C-4 position

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Summary. Under the action of the appropriate synthase from ripe tomatoes a 1:1 mixture of (3S,4R)-[3,4-$^2H_2$] and (3R,4S)-[3,4-$^2H_2$]-(2S)-adenosylmethionine is transformed into a 1:1 mixture of the two meso forms of [$^2H_2$]-1-aminocyclopropanecarboxylic acid, a result which proves the operation of an inversion mechanism and which is consistent with direct nucleophilic displacement of the leaving group in the substrate.

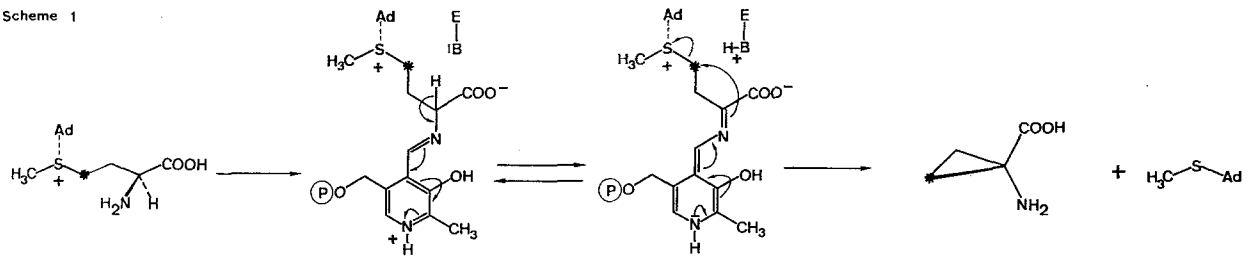
Key words. 1-Aminocyclopropanecarboxylic acid; ACC-synthase; PLP-catalyzed reaction; stereochemistry.

The amino acid 1-aminocyclopropanecarboxylic acid (ACC) plays an important role in higher plants as the precursor of the phytohormone ethylene[1,2]. Formation of ACC from S-adenosylmethionine (SAM) is catalyzed by a PLP-dependent synthase[9] and is expected to proceed according to scheme 1. Recently we have demonstrated that the Si-methylene group of ACC stems specifically from the C-4 methylene group of the substrate and thus that the reaction involves an inversion at the α-center[3]. To complete the stereochemical picture we decided to analyze the steric course of the substitution which takes place at the C-4 methylene group of the substrate.

An internal alkylation process involving direct displacement of methylthioadenosine is, of course, expected to proceed with inversion at the center of nucleophilic substitution. Alternatively, it is conceivable that the leaving group of the substrate is displaced by an appropriate nucleophile belonging to the protein component prior to the internal alkylation step, a process that would be expressed in an overall retention at the center undergoing the double substitution.

In principle the two possibilities can be distinguished by carrying out the enzymatic reaction with a [4-$^2H_1$]-SAM of known configuration at C-4 and determining the configuration at C-2 of the

Scheme 1

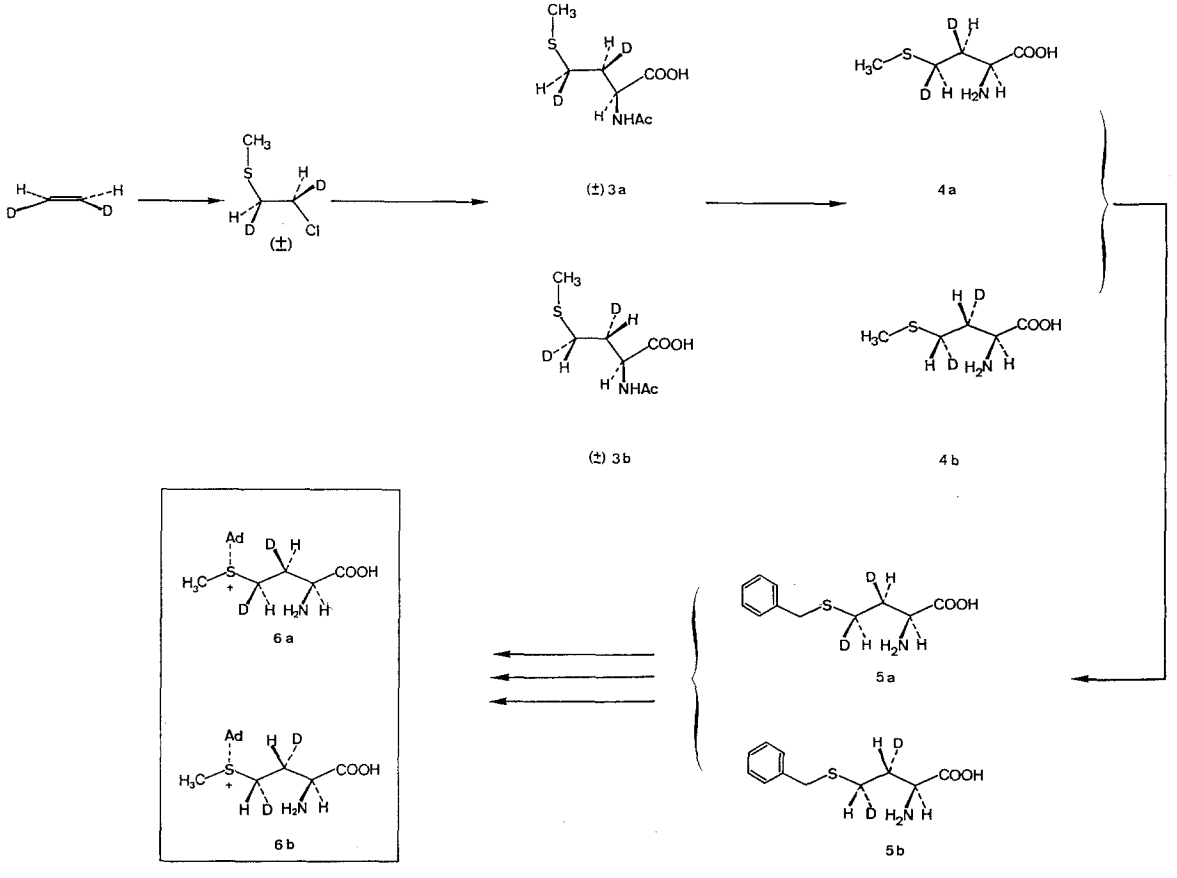


resulting (1S)-[2-$^2H_1$]-ACC. To avoid the problems posed by the preparation of the necessary bona fide reference specimen of monodeuteriated ACC, we elected instead to label the C-4 position of the substrate with one deuterium atom and to follow the stereochemical outcome of the reaction by using as an internal marker a second deuterium atom strategically placed at the C-3 position. This approach, which is well precedented[4], relies on knowledge of relative rather than absolute configuration and therefore allows the use of mixtures of stereoisomers possessing the same relative configuration at the deuteriated centers.

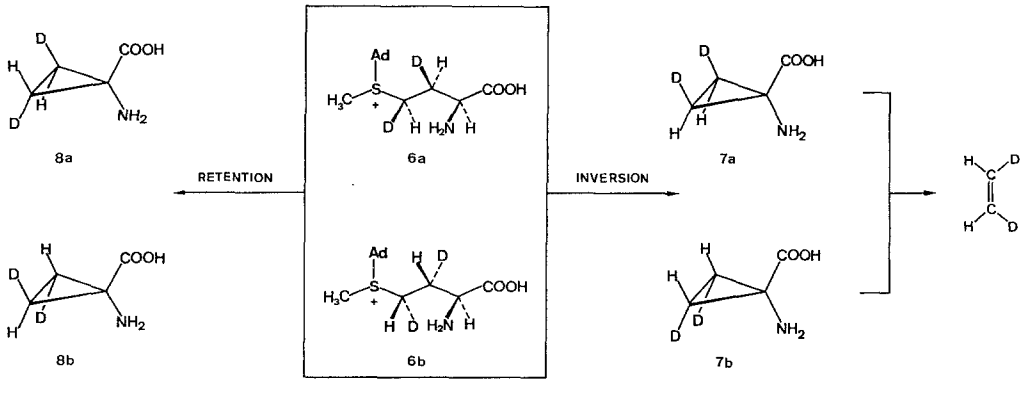
A mixture of the four dideuteriated *threo* stereoisomers of N-acetylmethionine, (±)3a and (±)3b, was prepared in three steps from (Z)-[1,2-$^2H_2$]-ethylene by the method of Billington and Golding[5] (scheme 2) and then treated with acylase to give the enantiomerically pure (2S) compounds (4a)+(4b). Attempted biological conversion of this material into SAM with baker's yeast[6] resulted in extensive and nonstereospecific loss of label from the C-3 position (> 85% as judged by NMR analysis of the isolated SAM specimen), probably brought about by rapid and reversible formation of the corresponding α-keto-acids. Eventually, the desired conversion of (4a)+(4b) into (6a)+(6b) was achieved by a known procedure[7] through the intermediacy of the S-benzylderivatives (5a)+(5b). In this way (6a)+(6b) were obtained in 40:60 admixture with the unnatural epimers at the sulfonium centers[8].

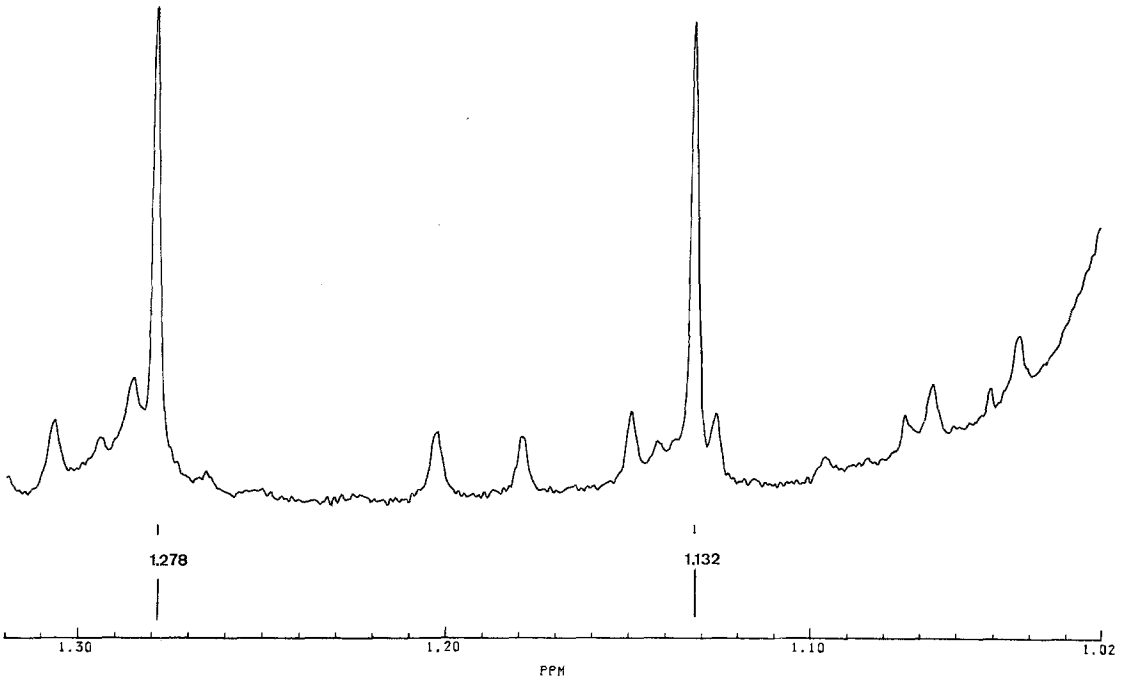
A sample of the labeled material containing 40 μmols of the natural substrate was allowed to react for 2 h with a crude extract of ACC synthase[9] prepared from 15 kg ripe tomatoes previously treated with lithium chloride[10]; after 2 h formation of 6 μmol of ACC was detected by the method of Lizada et al.[11]. The resulting mixture was freed from high molecular weight components by ultrafiltration and, after acidification, the amino acids were isolated by adsorption on an ion exchange resin column (Dowex 50, H^+) followed by elution with 1 M NH_4OH

Scheme 2



Scheme 3





Portion of the 300 MHz ^{1}H-NMR spectrum of the 1:1 mixture of the two meso forms *7a* and *7b* of [$^2H_2$]-Aminocyclopropanecarboxylic acid (D_2O, ^{2}H-decoupled, δ_{HOD} = 4.80). Some of the minor intensity signals coincide with the ^{13}-C-satellites expected from the presence of a large excess of valine (cf. text).

solution and then fractionated by chromatography on a cation exchange resin column with a linear HCl gradient. Fractions containing ACC, as detected by the method of Lizada et al.[11], were pooled and submitted to NMR analysis.

The presence of ACC as the only deuteriated species in this preparation was verified by the ^{2}H-NMR spectrum, which displayed the expected characteristic signals at δ 1.13 and 1.28[12]. The interpretation of the ^{1}H-NMR spectrum was complicated by the appearance of high intensity signals due to a 10-fold excess of valine and of a few additional low intensity signals caused by unidentified impurities. However, expansion of the critical region of the spectrum (fig.) clearly revealed the presence of two *singlets* of equal intensity at δ 1.13 and 1.28 belonging to the ACC from the enzymatic reaction. The absence of coupling in these two signals proves that they belong to different molecules and that each of them is due to a set of enantiotopically related protons. This is consistent only with the presence of a 1:1 mixture of the two meso forms (7a)+(7b) generated from the starting material through an inversion process (scheme 3). The alternative retention process would have led to the racemic form encompassing (8a)+(8b), which is known to exhibit in its ^{1}H-NMR spectrum two *doublets* with $J_{vicinal}$ = 7.7 Hz[13].

Confirmatory evidence for the meso configuration of the product of the enzymatic reaction was obtained by converting this compound in a stereocontrolled reaction with sodium hypochlorite[13] to a sample of cis-[$^2H_2$]-ethylene, which was identified through the characteristic IR signal at 842 cm^{-1} [14].

The inversion at C-4 detected in this work for the ACC synthase catalyzed reaction is in keeping with the direct substitution mechanism indicated in scheme 1. In this respect the intramolecular alkylation reaction which leads from SAM to ACC parallels the intermolecular nucleophilic substitutions of the same substrate catalyzed at C-4 by the enzyme spermidine synthase[15] and at the methyl group by different transmethylases[16,17].

Note added in proof: Independent results leading to the same conclusion have been published by K. Ramalingam et al.[18] after submission of this manuscript.

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