We propose that the use of these reagents should provide a valuable new approach to the determination of the structure of catalytic intermediates in solution. We are currently investigating derivatives **1b–g** (Y = B, Ga, Si; Z = C_6H_5, C_6D_5, which will provide internal standards so that (3 $\cos^2\alpha - 1$) for C-D and (3 $\cos^2\theta - 1)/r^3$ for Co–H, rather than relative values, can be determined.[21]

(21) We wish to acknowledge the support of the National Science Foundation for this research (CHE82-08042) and for its support of the NSF Northeast Regional NMR Facility (CHE79-16210).

Stereochemistry of the Enzymatic Ring Opening of 1-Aminocyclopropanecarboxylic Acid

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Received September 19, 1983

The amino acid 1-aminocyclopropanecarboxylic acid (ACPC), isolated from several plant tissues,[1] is an intermediate in the biosynthesis of the fruit-ripening hormone ethylene from methionine.[2] Pseudomonads can decompose ACPC to ammonia and 2-ketobutyrate via the PLP-linked enzyme ACPC deaminase.[3] From work in ^2H_2O it was shown that solvent protons are incorporated both at C-4 and, to some extent, at C-3 of the ketobutyrate, and this is consistent with a vinylglycyl-PLP imine intermediate[4] (Scheme I).

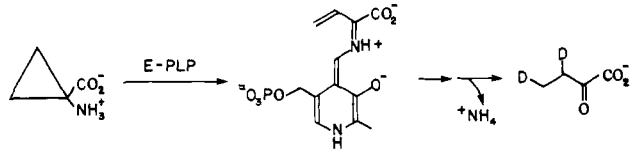
ACPC has two enantiotopic methylene groups, only one of which is expected to be processed by the deaminase to generate the methyl group of ketobutyrate. We have investigated this aspect of the reaction with specifically dideuterated ACPC specimens and herein report our results. The required substrates were prepared by two independent routes.

The Georgia synthesis began with 2,2-dichloro-1-phenylcyclopropanecarboxylic acid[5] (**3**), which was resolved with (+)- or (−)-α-methylbenzylamine to give the pure antipodes, $[\alpha]_D \pm 73°$ (CH_2Cl_2). For configurational assignment the enantiomerically pure methyl ester of (+)-**3** was reduced with 1.2 equiv of tri-*n*-butyltin hydride to give a mixture of monochloro esters from which the pure *Z* isomer **4**, mp 37–38 °C, $[\alpha]_D$ +190° (CH_2Cl_2), was isolated by chromatography[6] (Scheme II). $LiAlH_4$ reduction of **4**, followed by oxidation with pyridinium chlorochromate gave aldehyde **5**, $[\alpha]_D$ +211° (CH_2Cl_2), which, on treatment with Wilkinson's catalyst, afforded the known (1*S*,2*R*)-**6**, $[\alpha]_D$ +185° ($CHCl_3$).[8] The *R* configuration of (+)-**3** follows from the retention expected for the decarbonylation reaction.[9]

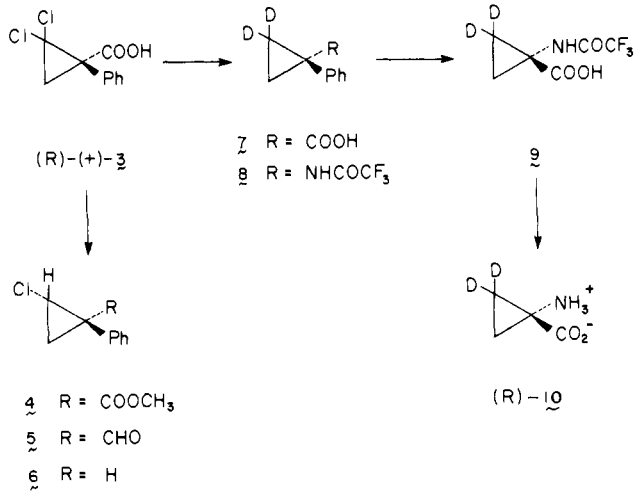
Reduction of (*R*)-**3** methyl ester with 2.5 equiv of tri-*n*-butyltin deuteride gave, after hydrolysis, the deuterated acid (*R*)-**7**, mp 84.5–85.5 °C, $[\alpha]_D$ +1.57° (CH_2Cl_2). Curtius rearrangement of **7** led to the amine, purified as the crystalline trifluoroacetamide **8**, mp 115–116 °C, $[\alpha]_D$ −1.28° (CH_2Cl_2). Oxidation of **8** with RuO_4 afforded acid (*R*)-**9**, mp 170 °C, $[\alpha]_D$ −0.90° (acetone), which was hydrolyzed to (*R*)-[2,2-$^2H_2$]ACPC (**10**). The *S* enantiomer of **9**, $[\alpha]_D$ +0.85°, was prepared from (−)-**3** in the same way and led to (*S*)-**10**.

In the Zürich synthesis acetate **11**, prepared from 3-methyl-3-butenoic acid by $LiAlD_4$ reduction followed by acetylation, was oxidized by SeO_2 and *tert*-butyl hydroperoxide to alcohol **12**, separated from concomitantly formed regioisomer by chromatography. Sharpless epoxidation[10] converted **12** to the *S* epoxide **13**. Reduction of an unlabeled specimen of **13** with $LiAlH_4$

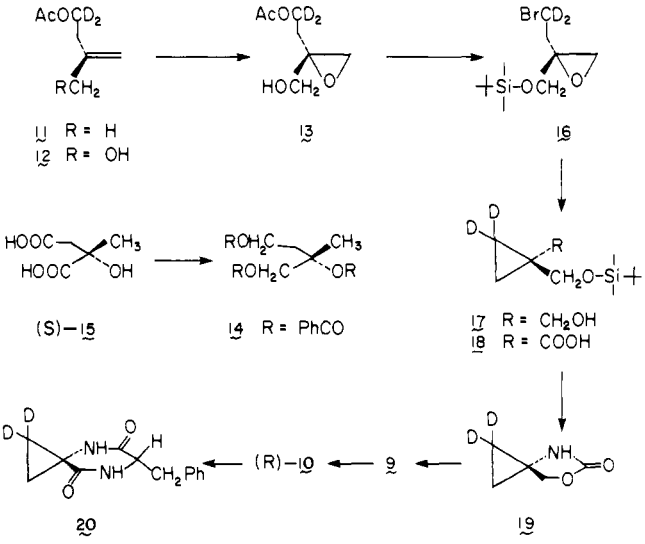
Scheme I



Scheme II



Scheme III



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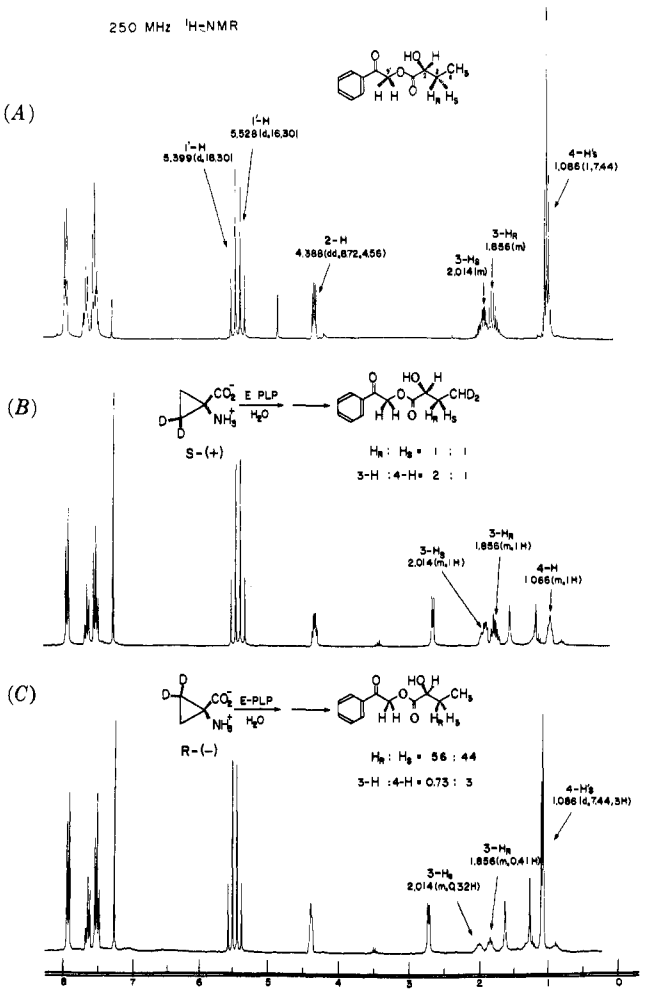

Figure 1. 250-MHz ^1H NMR of phenacyl esters from ACPC deaminase reactions: (A) standard, (B) incubation product from (*S*)-[$^2H_2$]-**10**, (C) incubation product from (*R*)-[$^2H_2$]-**10**.

followed by benzoylation gave the tribenzoate **14**, $[\alpha]_D$ +9.14° ($CHCl_3$). Comparison with authentic material, $[\alpha]_D$ +10.23°, prepared independently from (*S*)-citramalic acid (**15**)[11] confirmed the anticipated *S* configuration of **13** and established an enantiomeric purity of 90 ± 2%.

The *tert*-butyldimethylsilyl ether of **13** was converted in three steps (OH^-; TsCl; Bu_4NBr) into bromide **16**. The critical C–C bond was generated by an intramolecular displacement reaction when **16** was treated with lithium in THF to give the cyclopropane derivative **17**. Oxidation of **17** with RuO_4 to acid **18** and Curtius rearrangement of the latter furnished the oxazolidone **19**, which was converted by hydrolysis, trifluoroacetylation, and chromic acid oxidation to amide **9**, $[\alpha]_D$ −0.75° (acetone), subsequently hydrolyzed to (*R*)-(−)-**10**,[12] $[\alpha]_D$ −0.73° (*c* 3.5, H_2O); CD $\Delta\epsilon$ (205 nm) 4.13 × 10^{-3} L mol^{-1} cm^{-1} (H_2O) (Scheme III).

Enzymatic deamination of 6 mg of each labeled form of **10** was carried out as previously described,[4] coupling the ring opening with in situ reduction to (2*S*)-2-hydroxybutyric acid by NADH and L-lactate dehydrogenase. The resulting acid was converted to the crystalline phenacyl ester for NMR analysis. The NMR spectra in Figure 1 show unambiguously that ACPC deaminase differentiates sharply between enantiotopic methylene groups of its substrate. (*S*)-[$^2H_2$]ACPC (**10**) yields product deuterated exclusively in the methyl group (2.0 ^2H) whereas each of the independently synthesized *R*-[$^2H_2$] samples retains deuterium only in the product C-3 methylene group (1.27 and 1.29 ^2H, respectively). Thus, the methyl group of the product has its specific origin in the *pro-S* methylene of the substrate, and the regiospecificity of cleavage parallels that previously observed for the conversion of the higher homologue (1*S*,2*S*)-coronamic acid to 2-ketohexanoate.[13]

Partial loss of label (0.7 equiv), as in enzymic processing of (*R*)-[$^2H_2$]ACPC (**10**), is also observed in fragmentation of [$^2H_4$]ACPC (1.19 equivalents of ^2H at C-3 of product) and is not unexpected on the basis of earlier work with ACPC in ^2H_2O.[4] Accepting vinylglycyl-PLP imine equivalent (Scheme I) as a reaction intermediate,[4] this fractional exchange of label implies that the proton removed in the formation of that intermediate is shielded in the active site of the enzyme and therefore can return to C-3 in a subsequent step. The 56:44 distribution of label, evidenced in Figure 1C, among the stereoheterotopic positions at C-3 of the product indicates a nearly stererandom course for this reprotonation step.

Acknowledgment. This work has been supported in part by NIH Grants GM-28236 (R.K.H.) and GM 20011 (C.T.W.) and an NIEHS training grant (H.W.L.). Financial help from SANDOZ AG, Basel, is gratefully acknowledged. We thank Stephen E. Brown for large-scale resolutions and Richard Auchus for preparation of [$^2H_4$]ACPC.

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(12) For an independent assessment of the enantiomeric purity of the *R*-[$^2H_2$] acid, **10**, the compound was converted with (*S*)-phenylalanine to the diketopiperazine **20**, $[\alpha]_D$ + 71.9° (*c* 0.7, CH_3COOH). The ^1H NMR displayed two sharp doublets (δ 0.33 and 0.71, *J* = 5, 1 Hz) and two weak signals with less than 5% intensity (δ 0.95, 1.45) whereas in the ^1H spectrum of unlabeled **20**, the four methylene protons gave rise to four well-separated eight-line multiplets of equal intensity.

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Catalysis by Human Leukocyte Elastase. 2.[1] Rate-Limiting Deacylation for Specific *p*-Nitroanilides and Amides

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Received August 26, 1983

Serine proteases catalyze acyl-transfer reactions according to the kinetic mechanism of Scheme I.[2] In the absence of nucleophile, Nuc, k_c [$=k_2k_3/(k_2 + k_3)$] is commonly thought to be limited by deacylation, k_3, during ester hydrolysis and by acylation, k_2, during amide and anilide hydrolysis. Contrary to these generalities, Christensen and Ipsen have recently suggested[3] that reaction rates for highly specific peptide *p*-nitroanilides with certain serine proteases may be determined by deacylation and not acylation. Reported herein are steady-state kinetic experiments that address this problem for human leukocyte elastase. We report that *deacylation* is in fact rate limiting for the HLE[4]-catalyzed hydrolysis of specific-peptide esters, *p*-nitroanilides, and amides.

Comparisons of kinetic parameters for the HLE-catalyzed hydrolysis of peptide-derived substrates appear in Table I. Of

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(4) Abbreviations: MeOSuc, *N*-methoxysuccinyl; Ac, acetyl; pNA, *p*-nitroanilide; ONP, *p*-nitrophenyl ester; Phe-NH_2, L-phenylalaninamide; CHES, 2-(cyclohexylamino)ethanesulfonic acid; Hepes, *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; HLE, human leukocyte elastase.

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