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Synthesis of (±)-2,3-Methanoproline: A Novel Inhibitor of Ethylene Biosynthesis

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Abstract: The title compound, 2-*aza*-bicyclo[3.1.0]hexane-1-carboxylic acid (**2**) was prepared by treatment of N-benzyloxycarbonyl-2,3-dehydroproline *tert*-butyl ester with diazomethane followed by photolysis of the resulting pyrazoline and deprotection. Its N-acetyl-N'-methyl amide, a peptide mimic, was synthesized and the structure of was confirmed by X-ray diffraction studies. NMR spectroscopy was also used to examine the effect of the cyclopropane ring on its conformation. This 2,3-methanoamino acid (**2**) was found to be a weak inhibitor of ethylene biosynthesis in cucumber cotyledon strips and germinating squash seeds. The data show that **2** probably inhibits the conversion of 1-aminocyclopropanecarboxylic acid to ethylene in these tissues.

The biosynthesis of the plant hormone ethylene has attracted considerable interest in recent years particularly since 1-aminocyclopropanecarboxylic acid (Acc) was found to be the direct precursor of ethylene.[1] Much of the research in this area has concerned the mechanism by which Acc is degraded to ethylene, cyanide, and CO_2 by the ethylene forming enzyme (EFE)[2]. A second area of research has involved the search for potential inhibitors of EFE.[3] Early in these studies, Hoffman and coworkers[4] found that (+)-*allo*-coronamic acid (**1**, (1*R*, 2*S*)-2-ethyl-Acc) was converted into 1-butene at 25% the rate at which Acc yields ethylene, and that this stereoisomer of **1** was processed much more efficiently than any of the other stereoisomers (>40:1).

The many inhibitors of EFE tested have led various workers[3b,4] to propose a model for the active site of EFE (**Figure 1**) in which both the amino and carboxyl functions of Acc are required for binding to occur. The active site is also sterically constrained so that large groups at the 2-position cannot be accommodated.

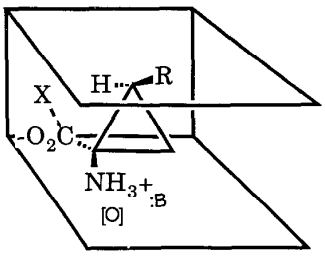
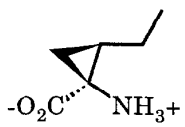


Figure 1



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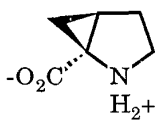


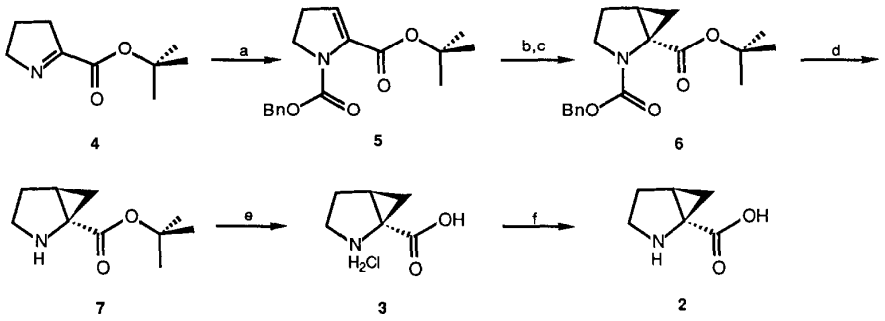
2

Figure 2

Based on this model, it was clear that 2,3-methanoproline (**2**, **Figure 2**) could be accommodated by the active site of EFE. This new cyclopropane amino acid would not only be an analog of proline but also of Acc; i. e., as a ***cyclo-allo***-coronamic acid, which would not be fully processed by EFE because it is a secondary amine. It might, therefore, be a tightly binding competitive or irreversible inhibitor of the enzyme.

Results and Discussion.

The synthesis of **2** was completed as shown (**Scheme 1**). Its hydrochloride salt (**3**) was prepared from the known[5] dehydroproline (**4**) in 31% overall yield. When this imine was treated with excess benzyl chloroformate and pyridine, the N-benzyloxycarbonyl derivative of enamine **5** was isolated and allowed to react with excess diazomethane. The intermediate pyrazoline was not isolated, but was directly photolyzed to give the fully protected cyclopropane **6**. Deprotection of **6** could be accomplished in a single step using trifluoroacetic acid and thioanisole or, more efficiently, in a two-step process involving hydrogenolysis (5% Pd/C) followed by acidolysis (2M HCl). The amino acid zwitterion was then obtained in 73% yield from the hydrochloride using Dowex-1 (acetate form).



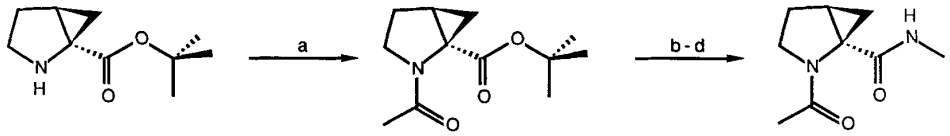
Reagents: a) CbzCl , Pyr ; b) CH_2N_2 ; c) hν ; d) H_2 , 5% Pd/C ; e) HCl; f) Dowex-1

Scheme 1

The crystalline N-acetyl-N'-methylamide derivative **8** was prepared from amino ester **7** by standard methods (**Scheme 2**). X-ray diffraction confirmed the structure of **8** (**Figure 3**) and, by inference, the structure of **2**. This particular derivative was chosen because it is a model peptide and its conformation should give an indication of the conformational behavior of this novel amino acid in peptides. The amide exhibited the small N2-C1-C2-N1 (ψ) dihedral angle which is characteristic of the conformations taken by 2,3-methanoamino acids in the solid state[6]. Furthermore, the ϕ and ψ angles of **8** are quite similar to those obtained by X-ray crystallography of the analogous proline derivative[7]; i.e., in amide **8** , ϕ = 76° and ψ = 7° while in N-acetylproline-N'-methylamide the corresponding values are 76° and 16°. The slightly smaller ψ angle in amide **8** is probably due to conjugation of the carbonyl group with the cyclopropane ring, which is maximized at $\psi = 0°$. These conformational similarities indicate that the cyclopropane containing amino acid may be an effective replacement for proline for purposes of stabilization to enzymolysis with retention of bioactivity.

Crystallography.

A crystal of $C_9H_{14}N_2O_2$ (**8**) was mounted on a Syntex P3 automated diffractometer. Unit cell dimensions (Table I) were determined by least squares refinement of the best angular positions for fifteen independent reflections ($2\theta>15°$) during normal allignment procedures using molybdenum radiation (γ=0.71069Å). Data, (1063 points) were collected at room temperature using a variable scan rate, a θ-2θ scan mode and a scan width of 1.2° below $K\alpha_2$ and 1.2° above $K\alpha_2$ to a maximum 2θ value of 45.0°. Backgrounds were measured at each side of the scan for a combined time equal to the total scan time. The intensities of three standard reflections were remeasured after every 97 reflections and as the intensities of these reflections showed less than 6% variation, corrections for decomposition were deemed unnecessary. Data were corrected for Lorentz, polarization and background effects. After removal of space group forbidden and redundent data, observed data, (423 points) ($I>3.0\sigma(I)$) were used for solution and refinement. The structures were solved for carbon, nitrogen and oxygen positions using direct methods[8]. Least squares refinement[9] converged with anisotropic thermal parameters. Hydrogen atoms were located from a difference Fourier synthesis. These positions were included in the final refinement with isotropic thermal parameters but held invariant. A final difference Fourier revealed no electron density of interpretable level. Scattering factors were taken from Cromer and Mann[10]. The final cycle of refinement - function minimized $\Sigma(|F_o|-|F_c|)^2$, led to final agreement factor, R=8.2%; $R=(\Sigma||F_o|-|F_c||/\Sigma|F_o|) \times 100$. Unit weights were used until the final cycles of refinement when weights equal to $1/\sigma F$ were introduced. R_w=10.8%.



7 8

Reagents: a) AcCl , NEt_3 b) TFA , CH_2Cl_2 c) $(CH_3)_2CHCH_2OCOCl$,NEt_3 d) $MeNH_3Cl$, NEt_3

Scheme 2

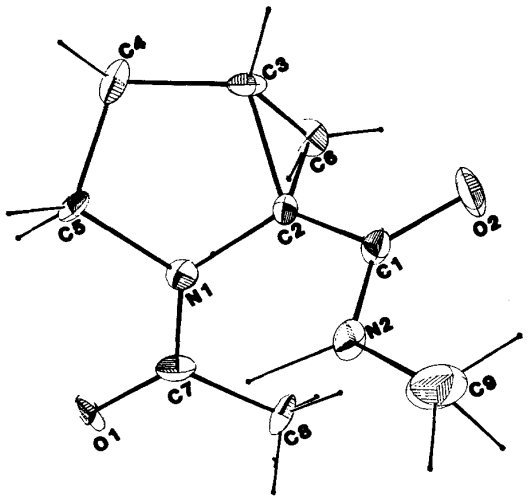


Figure 3

We have also carried out an NMR analysis of amide **8** in order to study its conformational properties in dilute solution and to compare them with the corresponding prolineamide derivative in order to allow interpretation of future peptide spectra containing the new amino acid. Using ^{1}H -^{1}H COSY, ^{1}H -^{13}C COSY, DEPT, steady-state NOE experiments, and a 500 MHz proton spectrum, we have been able to make preliminary assignments in D_2O of the protons and carbon atoms of both the s-*cis* and the s-*trans* conformers of **8** (**Figure 4**). In general, we found that **8** exhibits a somewhat greater preference for the s-*cis* conformation than does the prolineamide. In contrast, a recent study of N-acetyl-2,4-methanoproline-N'-methylamide by Scheraga[11] showed that only the s-*trans* conformer was present in dilute solution. Further NMR studies of amide **8**, now in progress, will be published separately.

Table 1. Ethylene production from plant tissues treated with (±)-2,3-methanoproline (**2**).

	Apple fruit cortex disks		Cucumber cotyledons		Squash seeds		Carrot roots
Concentration (mM)	Post-Climacteric	Pre-Climacteric	Strips	Whole	Germinating	Excised roots	Cortex disks
0.0	61.8[x]	1.61	1.52 a[z]	2.80	4.10 a	12.1	0.231
0.008	64.9	1.69					
0.024	65.0						
0.08	66.6	2.53			3.67 b	11.6	0.243
0.24	64.8						
0.8	61.7	2.04	1.19 b	2.39	2.87 c	13.0	0.252
2.4	61.3						
8.0	62.2	2.41	0.98 c	2.60	2.48 d	14.6	0.268
80			0.75 d	2.76			0.255
LSD 5%	n.s.	n.s.		n.s.		n.s.	n.s.

[x] Ethylene production in nl $(g \cdot hr)^{-1}$

[z] Means followed by the same letter are not significantly different at the 5% level.

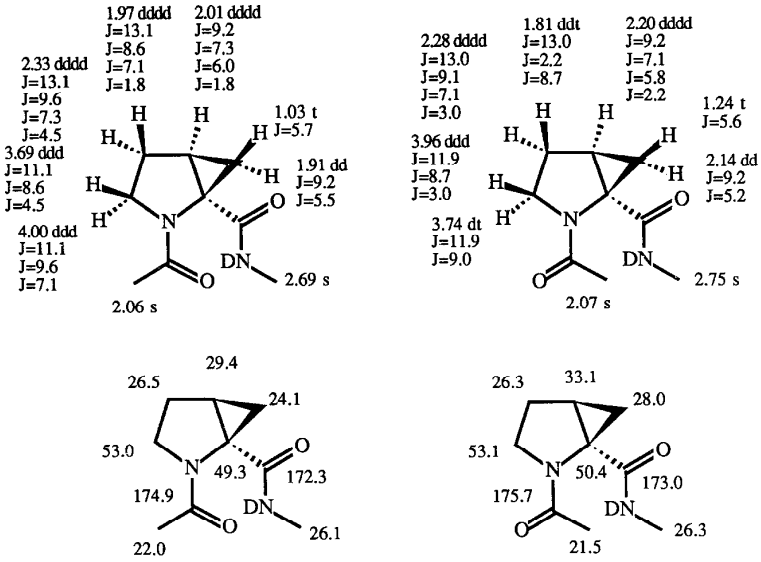


Figure 4

Biology

Application of 40 to 60 µl of 0.8 mM aqueous solutions of 2,3-methanoproline (**2**) to germinating squash seeds and strips of cucumber cotyledons reduced ethylene production by 30% and 20%, respectively. An 8 mM solution of **2** inhibited ethylene production by 36% and 40% in the same respective tissue. Inhibition was roughly loglinear and each 10-fold increase in concentration resulted in an additional 14% reduction in ethylene production from strips of cucumber cotyledons; i. e., 22%, 36%, and 50% reductions for 0.8, 8, and 80 mM solution, respectively.

In contrast, application of from 20 to 60 µl of up to 8 mM **2** had no inhibitory effect on ethylene production by cortex disks of pre- and post-climacteric apple tissue, carrot cortex tissue, whole cucumber cotyledons, or excised squash roots (**Table 1**). Ethylene production from whole cucumber cotyledons was unaffected by 20 µl of the 80 mM solution. Two applications of 20 µl of the 80 mM solution did result in a 28% reduction in ethylene production from pre-climacteric apple cortex disks, but this extremely high concentration was phytotoxic; treated tissue became brown and water soaked (data not shown).

Three other inhibitors of ethylene synthesis were more effective than **2**. Applications of 40 to 60 µl of 0.8, 8.0 and 80 mM solutions of **2** inhibited ethylene production in receptive tissue by an average of 26%, 38% and 50%, respectively, while application of 20 µl of a 10 mM solution of aminoethoxyvinylglycine (AVG) inhibited ethylene production

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