

2,4-Methanoproline (2-Carboxy-2,4-methanopyrrolidine)
and 2,4-Methanoglutamic Acid
(1-Amino-1,3-dicarboxycyclobutane) in Seeds of
Ateleia herbert smithii Pittier (Leguminosae)

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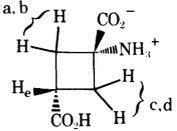
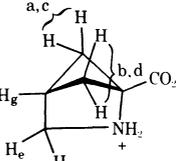
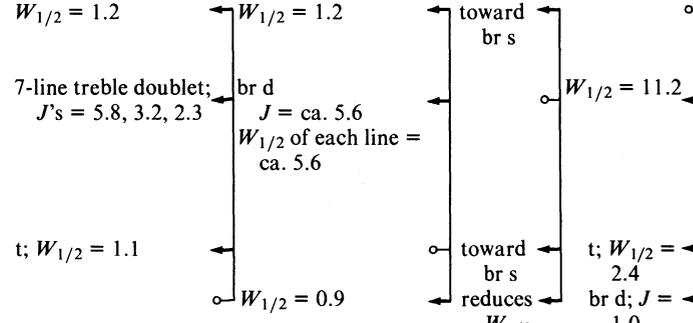
Abstract: A new acidic amino acid, 2,4-methanoglutamic acid, containing a cyclobutane ring and a new imino acid, 2,4-methanoproline, containing the previously unknown 2-azabicyclo[2.1.1]hexane system have been isolated from seeds of *Ateleia herbert smithii*. The structures of the two compounds were confirmed by X-ray crystallography. Unlike most amino and imino acids the isolated compounds do not show optical activity. Their possible biological significance is discussed.

The seeds of many legume species accumulate high concentrations of nonprotein amino acids and these may play a role in protecting the seeds from insect predation.^{2,3} Insects may, however, become adapted to diets containing potentially toxic amino acids as in the case of *Caryedes brasiliensis*, whose larvae live on canavanine-rich seeds.^{4,5}

The legume *Ateleia herbert smithii* Pittier, which is a locally common tree found in Costa Rica only in the vicinity of Santa Rosa National Park, Guancaste Province,⁶ produces seeds which are ignored by at least 100 seed predators in this habitat,

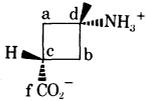
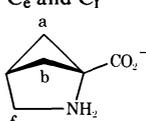
but are preyed on by the larvae of the weevil (Curculionidae) *Apion* sp. nov. In studying the chemistry of these seeds to determine whether the seeds contained secondary compounds likely to deter most predators, we found major concentrations of two acid-stable ninhydrin reacting compounds which could not be identified by their R_f values and ionic mobilities. Lower concentrations of a third ninhydrin-reacting "unknown" were also detected. The present paper describes the isolation and identification of the two major "unknowns" as 2,4-methanoproline and 2,4-methanoglutamic acid. Neither of these

Table I. 100-MHz ^1H NMR Spectra and Interpretation of the *Ateleia herbert smithii* Amino and Imino Acids for D_2O Solutions

comps and assignments	δ (from external Me_4Si); J , Hz; ^a $W_{1/2}$, Hz	double resonance experiments	
		(point of irrad)	point of effect (resulting change of signal, multiplicity, etc.)
 H's a, b, c, d He	2.68 m of 5 m lines 3.37 m of 5 br lines J 's = 9.1 and 9.5; $W_{1/2}$ = 2.6-4		
 Hc + Hd Ha + Hb Hg He + Hf	1.17 q (+ 2 sym side lines) J 's = 2.3, 6.2 (9.5); $W_{1/2}$ of each line = 1.6 2.31 br m; $W_{1/2}$ = 13 Hz 2.90 br t; J = 3.2; $W_{1/2}$ of each line = ca. 2.5 3.40 br s; $W_{1/2}$ = 2.8	$W_{1/2}$ = 1.2 7-line treble doublet; J 's = 5.8, 3.2, 2.3 t; $W_{1/2}$ = 1.1 t; $W_{1/2}$ = 1.0	 $W_{1/2}$ = 1.2 J = ca. 5.6 $W_{1/2}$ of each line = ca. 5.6 $W_{1/2}$ = 11.2 $W_{1/2}$ = 0.9 $W_{1/2}$ = 2.4 J = 1.0

^a Observed J 's are quoted for these chiefly non-first-order spectra.

Table II. 25-MHz ^{13}C NMR Spectra and Assignments of the *Ateleia herbert smithii* Amino and Imino Acids

compd (solvent)	δ_c (from external dioxane, 67.8 from Me_4Si) multiplicity in proton coupled spectra; J_{CH} , Hz
 (2 M NaOD/ D_2O) Cc Ca + Cb Cd Ce and Cf	34.0 d; J = 133 40.1 t; J = 135 56.2 s 184.8 s, 186.0 s
 (D_2O) Cd Ca + Cb Cf Cc Ce	38.0 d; J = 165 41.3 t; J = 147 50.4 t; J = 150 75.2 s 173.0 s

^a I was insufficiently soluble in D_2O , $\text{Me}_2\text{SO}-d_6$, and pyridine- d_5 .

compounds has been described before, but an isomer of the imino acid with a cyclopropane ring, L-*exo*-(*cis*)-3,4-methanoproline, occurs in *Aesculus parviflora*.⁷

Results and Discussion

Both compounds isolated from *A. herbert smithii* gave purple spots with ninhydrin on paper. The acidic compound gave a blue-purple and the neutral a green-blue spot on paper with isatin. The acidic compound formed a chelate with cupric ions indicating the presence of a free amino group in the 2 position.⁸ It gave R_f values of 17 when chromatographed on paper in 1-butanol-acetic acid- H_2O and 29 in phenol- H_2O in the presence of NH_3 . The neutral compound moved close to alanine in the same solvents. When subjected to electrophoresis on paper at pH 3.6 the acidic compound moved between aspartic acid and glutamic acid while the neutral compound was uncharged. At pH 1.9 the neutral compound carried a positive charge and moved faster than the acidic compound which ran with glutamic acid.

The molecular formula of the acidic compound was established as $\text{C}_6\text{H}_9\text{NO}_4 \cdot \text{H}_2\text{O}$ by microanalysis, EI and CI (NH_3 reagent gas) MS, and finally X-ray crystallography. The molecular formula of the neutral compound was established as $\text{C}_6\text{H}_9\text{NO}_2 \cdot \text{H}_2\text{O}$ by CIMS and X-ray crystallography. Elemental analysis and EIMS indicate, however, that the neutral compound can form a dihydrate which readily loses one H_2O on desiccation.

The EI mass spectra of both compounds showed the same basic peak at m/e 87 and many other fragment ions in common. ORD values for both compounds (measured from 200 to 589 nm) were zero.

Their IR spectra were typical of amino acids and indicated no other functional groups (ν_{max} KBr (cm^{-1}) acidic compound 3520, 2493, 3200-2350 br, 1683, 1580, 787, 755; neutral compound 3360 br, 3100-2350 br, 1650, 1580, 912, 895, 796, 751).

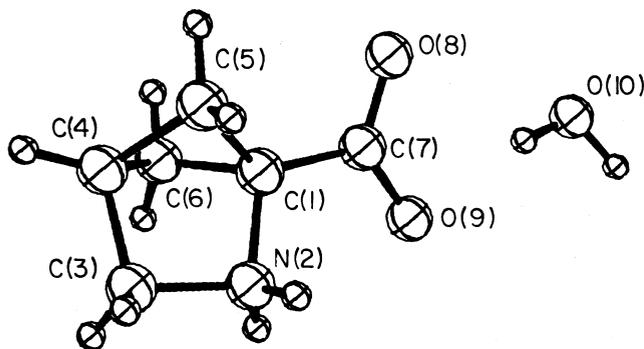


Figure 1. A computer-generated perspective drawing of the neutral imino acid.

The ^1H NMR spectra of both compounds were essentially non-first-order at 100 MHz (Table I) and showed a considerable amount of long-range couplings which in the case of the neutral compound were confirmed by double-resonance experiments. Their ^{13}C NMR spectra were considerably less complex (Table II).

Both compounds proved resistant to reduction by phosphorus and HI, suggesting cyclic rather than unsaturated structures.

The initial interpretation of the NMR data, together with the other data, indicated that both compounds were novel cyclobutane derivatives. This conclusion was supported by use of the computer structure generating program CONGEN⁹ and was confirmed by X-ray analysis. This X-ray analysis showed that the neutral imino acid crystallized in the orthorhombic crystal class with $a = 10.178(3)$, $b = 11.583$, and $c = 11.926(4)$ Å. Systematic extinctions uniquely indicated the common, achiral space group $Pbca$ and the density was consistent with one element of composition $\text{C}_6\text{H}_9\text{NO}_2 \cdot \text{H}_2\text{O}$ in the asymmetric unit. All unique diffraction maxima with $2\theta \leq 114$ were collected on a fully automated four-circle diffractometer using graphite monochromated $\text{Cu K}\alpha$ (1.541 78 Å) radiation and a variable speed ω scan. A total of 948 reflections were surveyed in this fashion and, after correction for Lorentz, polarization, and background effects 783 (83%), were judged observed ($F_o \geq 3\sigma(F_o)$). The structure was solved uneventfully by a multisolution sign determining procedure, and full-matrix least-squares refinements with anisotropic thermal parameters for nonhydrogen atoms and isotropic hydrogens have converged to a standard crystallographic residual of 0.053 for the observed reflections.¹⁰

Figure 1 is a computer-generated perspective drawing of the final X-ray model. The neutral imino acid is a derivative of the previously unknown 2-azabicyclo[2.1.1]hexane system. Bond lengths and location of the hydrogen atoms revealed that the molecule crystallizes as the zwitterion and there is also a molecule of H_2O in the asymmetric unit. The bicyclic fragment has approximate mirror symmetry but the plane of the carboxylate group is rotated 28° with respect to the plane defined by C(1), N(2), C(3), and C(4). The cyclobutane ring is folded with an angle of 46° between the planes defined by C(1), C(5), and C(6) and C(4), C(5), and C(6). There are three hydrogen bonds between symmetry-related molecules: N(2)H–O(8), 2.77 Å; N(2)H–O(10), 2.74 Å; O(10)H–O(9), 2.76 Å. There are no other short intermolecular contacts. Additional crystallographic details may be found in the supplementary material described at the end of this paper.

The acidic amino acid crystallizes in the triclinic crystal class with $a = 5.863(2)$ Å, $b = 8.141(3)$ Å, $c = 9.310(3)$ Å, $\alpha = 72.32(3)^\circ$, $\beta = 74.87(4)^\circ$, and $\delta = 75.22(4)^\circ$. A density measurement indicated two units of composition $\text{C}_6\text{H}_9\text{NO}_4 \cdot \text{H}_2\text{O}$ in the unit cell. Data were collected as described for the neutral amino acid and 944 (87%) of the 1086 reflections were

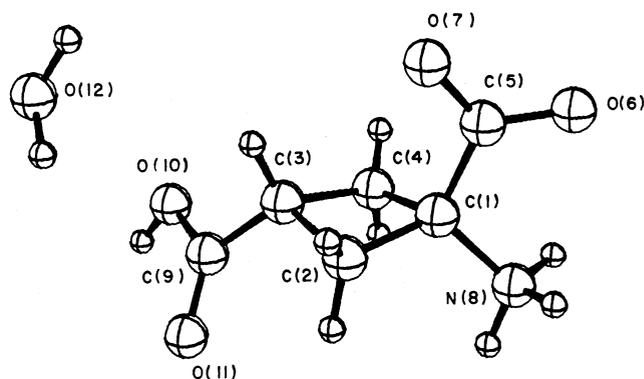


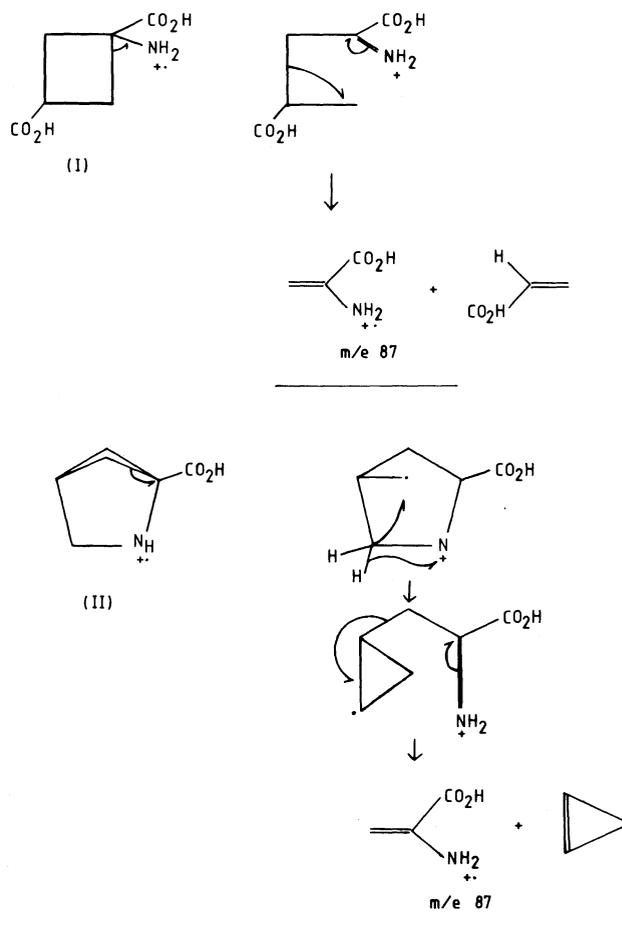
Figure 2. A computer-generated perspective drawing of the acidic amino acid.

judged observed. Solution and refinement in the centrosymmetric space group $P1$ was successful and the current crystallographic residual is 0.089 for the observed reflections.¹⁰

Figure 2 is a computer-generated perspective drawing of the final X-ray model of 1-amino-1,3-dicarboxycyclobutane plus the H_2O of crystallization. The material crystallizes as a zwitterion with the substituents at C(1) bearing the charges. The cyclobutane ring is only slightly folded with an interplanar angle of $\sim 15^\circ$. There is an extensive intermolecular hydrogen bonding network: N(8)H–O(7), 2.75 Å; O(12)H–O(11), 2.79 Å; O(10)H–O(6), 2.58 Å; N(8)H–O(12), 2.76 Å; N(8)H–O(6), 2.86 Å; O(12)H–O(11), 2.87 Å. There are no other short intermolecular contacts and additional details are available in the supplementary material.

From these structures the origin of the common peak at m/e 87 ($\text{C}_3\text{H}_5\text{NO}_2$) in the EI mass spectra can be rationalized in terms of fragmentations shown in Scheme I.

Scheme I



Experimental Section

Isolation of 2,4-Methanoproline. Finely ground mature seed of *Ateleia herbert smithii* (119 g) was extracted six times at room temperature with 75% ethanol (200 mL). After filtration the combined extracts were concentrated to 150 mL under reduced pressure and applied to a column (75 × 3 cm) of cation-exchange resin (Dowex 50 × 8) in the H⁺ form. The column was washed with water until the effluent was colorless. The neutral and acidic amino acids were then displaced with 1 N pyridine and the effluent was collected in 10-mL fractions. The ninhydrin-reacting fractions (40–170) were combined and concentrated under reduced pressure and the concentrate (50 mL) was applied to a column (75 × 3 cm) of anion-exchange resin (Amberlite IR-45) in the OH⁻ form. The neutral amino acids were displaced from the column with water and those fractions containing the neutral "unknown" were taken to dryness in a rotary evaporator at <40 °C. The residue was triturated with boiling ethanol and water added drop by drop with continued heating until the residue was dissolved. The hot solution was filtered and on cooling crystals separated from the filtrate. These were recrystallized three times from aqueous ethanol; yield 0.3 g. Anal. (C₆H₁₃NO₄) C, H, N.

Isolation of 2,4-Methanoglutamic Acid. After the neutral amino acids were removed from the column of anion-exchange resin with water, with the acidic amino acids were displaced with 1 N acetic acid and the fractions containing the "unknown" acidic compound (as determined by electrophoresis) were taken to dryness under reduced pressure. The residue was recrystallized from water, yield 0.3 g. Anal. (C₆H₁₁NO₅) C, H, N.

HVPE. Electrophoresis was carried out on paper at pd of 75 V/cm using buffer solutions of pH 1.9 and 3.6.¹¹

Two-Dimensional Chromatography. The ascending technique was employed using Whatman no. 1 paper. Solvents were 1-butanol-acetic acid-water (12:3:5 by vol) and phenol-water (4:1 w/v) in the presence of NH₃ (0.88) vapor.

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Supplementary Material Available: Tables of fractional coordinates (Table 1), bond distances (Table 2), bond angles (Table 3), and observed and calculated structure factors for both the neutral imino acid and the acidic amino acid (10 pages). Ordering information is given on any current masthead page.

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