*Tetrahedron* Vol. 43, No. 8, pp. 1857 to 1861, 1987
Printed in Great Britain.

0040-4020/87 $3.00+.00


# ISOLATION FROM ATELIA HERBERT-SMITHII PITTIER (SOPHOREAE, LEGUMINOSAE) AND X-RAY STRUCTURE OF CIS-1-AMINO-3-HYDROXYMETHYL-CYCLOBUTANE-1-CARBOXYLIC ACID, AN ACHIRAL NON-PROTEIN AMINO ACID

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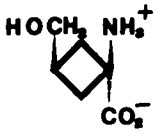
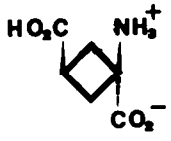
(*Received in UK 6 February* 1987)

cis-1-Amino-3-hydroxymethyl-cyclobutane-1-carboxylic Acid (1) has been isolated from Atelia herbert-smithii Pittier (Leguminosae) and its structure determined by spectroscopic and X-ray crystallographic methods. By a study of the $^1$H NMR spectrum of the crude extract, the relative amount of (1) to that of methanoproline in the plant was shown to be 1 to 1.15.

There are few naturally occurring 1-aminocycloalkane-1-carboxylic acids;[1] for example, 1-amino-2-nitrocyclopentane-1-carbocylic acid from Aspergillus wenttii[2] and 1-aminocyclopropane-1-carboxylic acid, an intermediate in ethylene biosynthesis.[3] Atelia (DC.) Benth. is a primitive neotropical legume genus of 16 species.[4] It is distributed from Mexico through northern Central America, the West Indies and into South America. Atelia herbert-smithii Pittier[5] is a medium sized dioecious tree now known from only two areas: Leon Department in Nicaragua and the Santa Rosa National Park in Costa Rica. Recently, a chemical investigation of seeds of Atelia herbert-smithii led to the isolation of two achiral 1-aminocyclobutane-1-carboxylic acids: one, an acidic compound, was identified as 2,4-methanoglutamic acid (2) and the other, a neutral amino acid, was shown to be 2,4-methanoproline (3).[6] This paper describes the isolation and characterisation of a third achiral cyclobutane amino acid, cis-1-amino-3-hydroxymethyl-cyclobutane-1-carboxylic acid (1).




(1)

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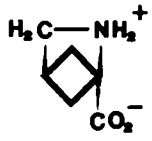


(3)

$^1$H NMR spectra of samples of 2,4-methanoproline (3), isolated by ion exchange chromatography[6] from seeds of Atelia herbert-smithii Pittier showed the presence of an impurity. This impurity could be separated from (3) by applying 0.5 g of this mixture to an alumina column (40 x 1.5 cm) made up in ethanol; the new compound could be eluted from the column with 95% aqueous ethanol under conditions where (3) remained on the column. Removal of the solvent under reduced pressure gave a residue which was recrystallised from aqueous ethanol to give cis-1-amino-3-hydroxymethyl-cyclobutane-1-carboxylic acid (1) (0.2 g) as the hydrate (Found C, 43.84; H, 7.86; N, 8.58. $C_6H_{11}O_3N.H_2O$ requires C, 44.17; H, 7.60; N, 8.59%).

The amino acid (1) decomposed at 210°C without melting. The CI mass spectrum gave a base peak at m/z 146 ($M+H^+$), together with fragment peaks at 128 ($M-OH^+$, 35%), 100 ($M-COOH^+$, 25%) and 87 [$CH_2=C(NH_2)COOH^+$, 35%]; the peak at 87 has been reported as the base peak in the mass spectra of both methanoproline (3) and of methanoglutamic acid (2). The infra red spectrum was typical of an amino acid and indicated no other functional group [3600 - 2500 (OH, NH), 1640, 1620 (C=O) $cm^{-1}$]. The $^1H$ spectrum was non-first order and showed a significant amount of long range coupling: δ 3.45 (2H, d, J=5.7 Hz, $C\underline{H}_2OH$), 2.55-2.40 (3H, m, $C\underline{H}.CH_2OH$ and 2 x $C\underline{H}_A\underline{H}_BCNH_2$), and 1.94 (2H, dd, J=11.0 and 7.5 Hz, 2 x $CH_A\underline{H}_BCNH_2$). In contrast, the $^{13}C$ NMR spectrum was simple: δ 177.6 (s, COOH), 65.8 (t, $CH_2OH$), 55.8 (s, $CNH_2$), 33.5 (t, $CH_2$), and 30.2 (d, CH). The cis-stereochemistry of the relationship between the hydroxymethyl group and the amino group was established by X-ray crystallographic analysis of the hydrochloride of the amino acid (Figure), crystallised from aqueous ethanol; crystals obtained of the neutral amino acid were not suitable for X-ray investigation.

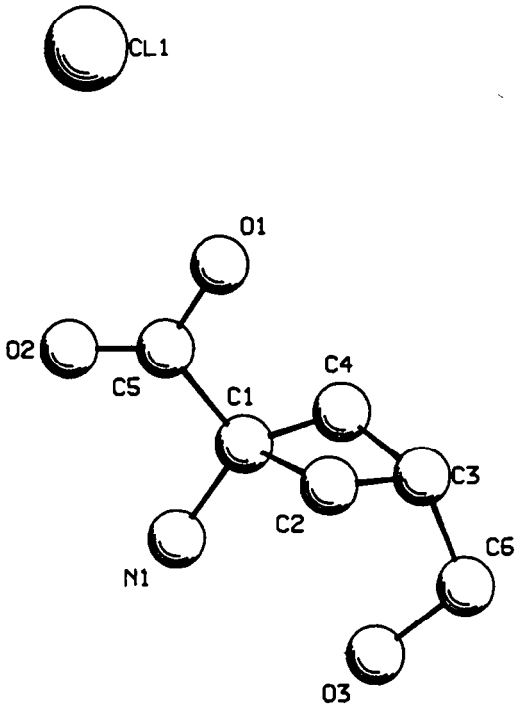


Figure. X-Ray Molecular Structure of cis-1-Amino-3-hydroxymethyl-cyclobutane-1-carboxylic Acid Hydrochloride with the crystallographic numbering scheme.

The $^1H$ NMR spectrum of a mixture of (1) and methanoproline (3) allows an estimate to be made of the relative amounts of the two amino acids in the crude extract by integration of the high field protons in the two compounds; the ratio of (3) to (1) as determined by this procedure is approximately 1.15 to 1. From 721 g of seed,

yields of 1% of each of (1) and (3), and 0.7% of (2), were obtained. The seeds of four other Atelia species and of both species of the closely related genus Cyathostegia contained levels of (1), (2) and (3) estimated from ionophoresis papers to be similar to those found in seeds of A. herbert-smithii; the three cyclobutane amino acids were also major components of the free amino acids in the leaves of these species.

The 3-substituents in (1), (2) and (3) are all trans to the 1-carboxyl group, indicating that the compounds may be metabolically related. No synthesis of 2,4-methanoglutamic acid has been reported; two syntheses of 2,4-methanoproline, in which the key steps are [2+2] cycloadditions, have appeared.[7,8]

Acknowledgements. We are indebted to Dr. C. K. Prout for advice and help in this study. We also thank SERC (H.-F.C) and the Bentham-Moxon Trust (R. J. N.) for financial support.

## EXPERIMENTAL

Examination of plant extracts for free amino acids. Finely ground seeds of Atelia herbert-smithii, A. apetala Griseb, A. glaziovii Baill., A. cubensis Griseb, A. arsenii Standley, Cyathostegia matthewsii Schery and C. weberbauerii Schery were extracted with 75% aqueous ethanol (200 mg/ml). The extracts were analysed by high voltage ionophoresis on Whatman No. 1 paper (70 V/cm for 30 min in buffer solutions of pH 1.9 and 3.6. A partial separation was acheived at pH 1.9, (3) having a slightly lower mobility than (1), whereas at pH 3.6, the ionic mobilities were identical. (1) gave a purple colour with ninhydrin reagent (0.2% w/v in acetone) and a green/blue colour with isatin reagent (0.2% w/v in 4% acetic acid in acetone) on paper. (3) reacted weakly with ninhydrin to give a grey colour and did not react with the isatin reagent; it did however give a blue colour with aqueous sodium nitroprusside (10%) - acetaldehyde (10%) - sodium carbonate (5%) (1:1:2). A voucher specimen of A. herbert-smithii is deposited in the herbarium at the Royal Botanic Gardens at Kew (Tanyen 1978 s.n. Kew).

Structure Determination. The infra red spectrum was recorded as a KBr disc on a Perkin-Elmer 781 spectrophotometer. The $^{1}H$ NMR spectra were run in $D_2O$ at 300 MHz on a Bruker WH 300 spectrometer and the $^{13}C$ NMR spectrum was run in $D_2O$ at 62.9 MHz on a Bruker AM 250 spectrometer, using 1,4-dioxan ($\delta$ 67.6) as the internal standard. The microanalysis was performed by the microanalytical service of University College London. The mass spectrum was measured on a VG Micromass 30F spectrometer, using the desorption chemical ionisation (DCI, $NH_3$) technique. The melting point was recorded on a Kofler block.

X-Ray Crystal Structure Analysis. The crystal data for the hydrochloride of cis-1-amino-3-hydroxymethyl-cyclobutane-1-carboxylic acid are given in Table 1. The crystals were recrystallised from aqueous ethanol. X-Ray data were collected with an Enraf-Nonius CAD4 diffractometer following the procedures recommended in the manufacturer's manual. The data were corrected for Lorentz and polarisation effects and for absorption. All calculations were carried out on a VAX 11/750 computer using SHELXS-86[9] for direct methods and CRYSTALS[10] for all other calculations. Atomic scattering factors were taken from International Tables.[11] The positions of all non-hydrogen atoms were given by the SHELXS-86 direct methods routine. A difference map revealed the positions of the OH and NH hydrogen atoms and the CH hydrogen atoms were placed geometrically. The trial structure was refined by full matrix least squares with isotropic temperature factors for the hydrogen atoms and anisotropic temperature factors for all other atoms. The Figure shows the molecular structure of the hydrochloride of cis-1-amino-3-hydroxymethyl-cyclobutane-1-carboxylic acid with the crystallographic numbering scheme. The atomic coordinates have been deposited at the Cambridge Crystallographic Data Centre.

Table 1. Crystal Data for the Hydrochloride of cis-1-Amino-3-hydroxymethyl-cyclobutane-1-carboxylic Acid.

| | |
|---|---|
| Formula | $C_6H_{12}ClNO_3$ |
| Mr | 181.6187 |
| crystal size/mm | 4 x 3 x 2 |
| crystal class | orthorhombic |
| a/Å | 9.004(6) |
| b/Å | 11.094(8) |
| c/Å | 17.197(1) |
| α/° | 90 |
| β/° | 90 |
| γ/° | 90 |
| Space group | $P_{cab}$ |
| Z | 8 |
| $D_c$/g cm$^{-3}$ | 1.4037 |
| F(000) | 768 |
| U/Å$^3$ | 1718.062 |
| Radiation | CuKα |
| μ/cm$^{-1}$ | 18.5871 |
| $(\sin\theta/\lambda)_{max}$ | 0.63246 |
| [a]Total I | 2700 |
| [b]unique I | 1237 |
| Rm/10$^{-2}$ | 3.35 |
| [c]n | 3 |
| R/10$^{-2}$ | 4.14 |
| Rw/10$^{-2}$ | 5.79 |
| [d]shift/error | 0.00 |
| [e] max/eÅ$^{-3}$ | 0.05 |
| Ext para | 24.2 |
| [f]Weights | 17.2, 12.4, 10.8 |

[a] total number of reflections measured. [b] number of unique reflections with intensity significantly above the background intensity. [c] criterion for recognising observed reflections $I > n\sigma(I)$. [d] ratio of maximum least-squares shift to error in final refinement cycle. [e] maximum height in final difference electron density synthesis. [f]Three-term Chebychev weighting scheme[12]

Table 2. Fractional atomic coordinates and equivalent* isotropic temperature factors with e.s.d's in parenthesis (atomic labelling as in Figure) for the Hydrochloride of cis-1-Amino-3-hydroxymethyl-cyclobutane-1-carboxylic Acid.

| Atom | x/a | y/b | z/c | U(equiv) |
|---|---|---|---|---|
| Cl(1) | 0.12962(7) | 0.96793(5) | 0.35366(4) | 0.0383 |
| O(1) | 0.1070(3) | 0.6948(2) | 0.3720(2) | 0.0519 |
| O(2) | 0.2873(2) | 0.6757(2) | 0.2838(1) | 0.0470 |
| O(3) | 0.2534(2) | 0.7280(2) | 0.1090(1) | 0.0426 |
| N(1) | 0.3090(2) | 0.4364(2) | 0.3032(1) | 0.0352 |
| C(1) | 0.2070(3) | 0.5021(2) | 0.3560(1) | 0.0319 |
| C(2) | 0.0567(3) | 0.4355(2) | 0.3653(2) | 0.0366 |
| C(3) | 0.1066(3) | 0.3885(2) | 0.4452(2) | 0.0356 |
| C(4) | 0.2407(3) | 0.4758(2) | 0.4424(1) | 0.0365 |
| C(5) | 0.2060(3) | 0.6338(2) | 0.3316(2) | 0.0370 |
| C(6) | 0.1472(4) | 0.2568(2) | 0.4500(2) | 0.0414 |

* $U_{eq} = (U_1, U_2, U_3)$ where the mean-square displacements (Å$^2$) are along the principal axes of the thermal ellipsoid.

Table 3. Bond lengths (A) for the non-hydrogen atoms with e.s.d.'s in parenthesis (atomic labelling as in Figure) for the Hydrochloride of cis-1-Amino-3-hydroxymethyl-cyclobutane-1-carboxylic Acid.

| | | |
|---|---|---|
| O(1) | C(5) | 1.317(3) |
| O(2) | C(5) | 1.194(3) |
| O(3) | C(6) | 1.429(3) |
| N(1) | C(1) | 1.484(3) |
| C(1) | C(2) | 1.550(3) |
| C(1) | C(4) | 1.545(3) |
| C(1) | C(5) | 1.520(3) |
| C(2) | C(3) | 1.537(4) |
| C(3) | C(4) | 1.547(4) |
| C(3) | C(6) | 1.508(3) |

Table 4. Bond angles (°) for the non-hydrogen atoms with e.s.d.'s in parenthesis (atomic labelling as in Figure) for the Hydrochloride of cis-1-Amino-3-hydroxymethyl-cyclobutane-1-carboxylic Acid.

| | | | |
|---|---|---|---|
| C(2) | C(1) | N(1) | 111.7(2) |
| C(4) | C(1) | N(1) | 112.0(2) |
| C(4) | C(1) | C(2) | 89.0(2) |
| C(5) | C(1) | N(1) | 107.3(2) |
| C(5) | C(1) | C(2) | 118.8(2) |
| C(5) | C(1) | C(4) | 116.6(2) |
| C(3) | C(2) | C(1) | 89.9(2) |
| C(4) | C(3) | C(2) | 89.4(2) |
| C(6) | C(3) | C(2) | 116.6(2) |
| C(6) | C(3) | C(4) | 114.9(2) |
| C(3) | C(4) | C(1) | 89.7(2) |
| O(2) | C(5) | O(1) | 125.3(2) |
| C(1) | C(5) | O(1) | 110.6(2) |
| C(1) | C(5) | O(1) | 124.0(2) |
| C(3) | C(6) | O(3) | 109.8(2) |

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