The Identification of a Hydroxylated Pyrrolidine Derivative from *Castanospermum australe*

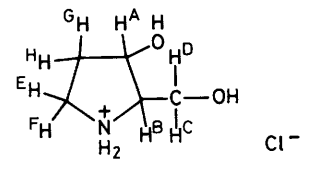
Robert J. Nash,[a] E. Arthur Bell,*[a] George W. J. Fleet,[b] Richard H. Jones,[b] and J. Michael Williams *[c]

[a] *Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey TW9 3DS, U.K.*
[b] *Chemical Crystallographic Laboratory, Oxford University, 9 Parks Road, Oxford OX1 3PD, U.K.*
[c] *Chemistry Department, University College, Singleton Park, Swansea SA2 8PP, U.K.*

A new pyrrolidine derivative, (2*R*,3*S*)-2-hydroxymethyl-3-hydroxypyrrolidine, has been isolated from *Castanospermum australe*, and identified by n.m.r. and mass spectroscopy and *X*-ray crystallography.

The β-glucosidase inhibitor 1,6,7,8-tetrahydroxyoctahydroindolizine (castanospermine) occurs in the seeds of the legume *Castanospermum australe* A. Cunn.[1,2] We report here the isolation of a novel hydroxylated pyrrolidine derivative from the same source.

The alkaloid was isolated as a crystalline hydrochloride, m.p. 108—112 °C, $[\alpha]_D^{21}$ + 46.5° (H_2O), after chromatography of a 75% aqueous ethanolic extract of the seeds on cation and anion exchange resins. Its composition was shown to be $C_5H_{12}ClNO_2$ by elemental analysis and mass spectrometry, the protonated amine being detected at *m/z* 118 in the chemical ionisation mass spectrum. The ^{13}C and ^{1}H n.m.r.



(1)

Coupling constants J/Hz

AB	3.9	EF	−11.8
AG	3.95	EG	5.5
AH	5.9	EH	8.75
BC	4.2	FG	7.4
BD	8.0	FH	8.15
CD	−12.2	GH	−14.0

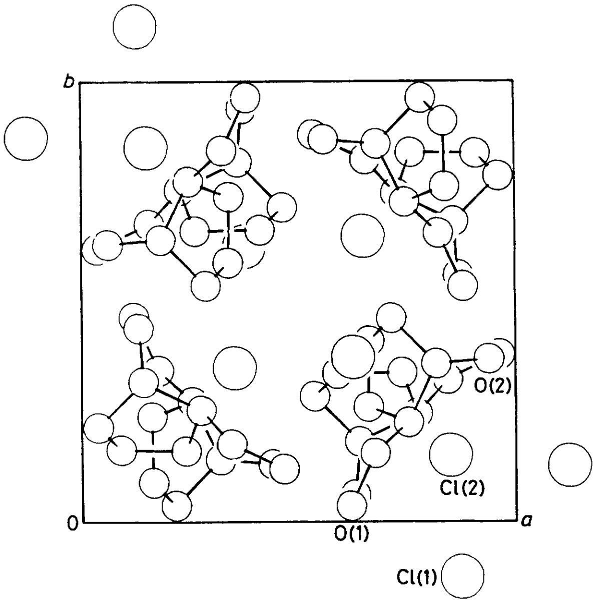


Figure 1. Crystal packing in the unit cell, viewed along *c* and showing the alternative positions of the chloride ion (hydrogen atoms omitted for clarity).

spectra (for a D_2O solution) established the structure as 2-hydroxymethyl-3-hydroxypyrrolidine hydrochloride (**1**). The 300 MHz ^1H spectrum contained multiplets for eight protons which included three geminally coupled pairs (CH_2 groups). The signal at lowest field (δ 4.24) in the ^1H spectrum was assigned to the proton at C-3; this proton was shown by spin-decoupling to be adjacent to a CH_2 group, which gave signals at highest field (δ 2.13 and 1.90), and to one other proton which resonated at δ 3.47. Complete analysis of the multiplets was achieved using spin simulation;† the vicinal and geminal coupling constants are given. The structure (**1**) was supported by the electron impact mass spectrum which contained, in addition to a weak molecular ion for the free amine, a very intense ion at m/z 86 which resulted from loss of CH_2OH.

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† The SIMEQ II programme of C. W. F. Kort and M. J. A. de Bie was used on a Varian L100 computer.

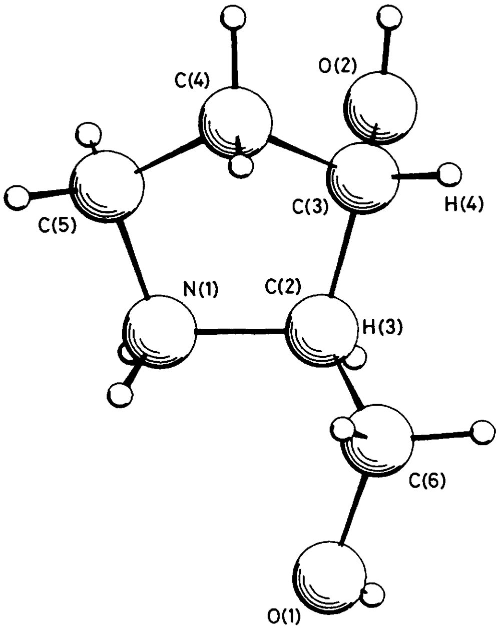

Figure 2. Molecular structure of compound (**1**).

The relative and absolute configurations were determined by *X*-ray crystallography.‡ *Crystal data*: $C_5H_{12}ClNO_2$, tetragonal, space group $P4_12_12$, $a = 9.940(3)$, $c = 14.822(2)$ Å, $U = 1464.59$ Å^3, $D_c = 1.393$ g cm^{-3}, $Z = 8$, $F(000)$ 656, μ(Mo-K_α) = 4.54 cm^{-1}. The crystal structure is interesting in that the chloride ion is found on two *independent* positions, instead of

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‡ Cell parameters and reflection intensities were obtained using graphite-monochromated Mo-K_α radiation on an Enraf–Nonius CAD-4 diffractometer operating in the ω–2θ scan mode for a crystal having approximate dimensions 0.23 × 0.55 × 0.13 mm. The scan range (ω) was calculated from $[1.00 + 0.35 \tan\theta]$, and the scan speed varied from 1.0 to 6.7 min depending upon the intensity. 4948 Reflections were scanned in the range $0 < \theta < 25°$. Friedel pairs were measured sequentially so as to minimise instability effects in the system. Three intensity control reflections measured every hour showed a gradual decrease with time (*ca.* 11.6%) and appropriate scaling factors were applied to reflections. The data were corrected for Lorentz, polarisation, and absorption effects[3] (relative transmission factors 0.99, 1.09); equivalent reflections were merged ($R_m = 0.015$) to give 1227 reflections (including Friedel pairs) of which 1181 were considered to be observed $[I > 3\sigma(I)]$ and used in the structure analysis.

The structure was solved by the direct methods package MITHRIL[4] and refined initially with isotropic and subsequently with anisotropic temperature factors for the heavier atoms. Hydrogen atoms were located in difference Fourier maps and their positional parameters were included in the refinement. The isotropic temperature factors of the hydrogen atoms were set at 1.2 times that of the atom they were bonded to. Restraints[5] were applied to the bond lengths involving hydrogen atoms. In the final stages of refinement an enantiopole parameter was included[6] and refined to −0.017(87) showing that the molecule depicted in Figure 1 has the correct absolute stereochemistry. The weight for each reflection in the final cycles of refinement was calculated[7] from the Chebyshev series $w = [68.89\, t_0(X) + 89.74\, t_1(X) + 22.95\, t_2(X)]^{-1}$ where $X = F_0/F_{\text{max.}}$. The structure converged at a final R value of 0.025 ($R_w = 0.032$). All calculations were performed with the CRYSTALS[8] package of programs on the Chemical Crystallography VAX 11/750 computer. The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

occupying one position, thereby maximising hydrogen bonding (see Figure 1). The O(1)–H(11) · · · Cl(1) and O(2)–H(12) · · · O(1) hydrogen bonds join the molecules into sheets perpendicular to *c*; these sheets are linked by the N(1)–H(2) · · · Cl(2) hydrogen bond. Compound (**1**) was thus shown to be (2*R*,3*S*)-2-hydroxymethyl-3-hydroxypyrrolidine hydrochloride and the configuration is shown in Figure 2.

Other hydroxylated pyrrolidine derivatives (excluding amino acids) found in nature include 2,5-dideoxy-2,5-imino-L-mannitol,[9] isolated from the leaves of *Derris elliptica*, and the 2-hydroxymethyl-3,4-dihydroxypyrrolidine recently isolated from *Angylocalyx boutiqueanus*.[10] The biological activity of compound (**1**) will be reported later.

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