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CASTANOSPERMINE, A 1,6,7,8-TETRAHYDROXYOCTAHYDROINDOLIZINE ALKALOID, FROM SEEDS OF *CASTANOSPERMUM AUSTRALE*

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Key Word Index—*Castanospermum australe*; Leguminosae; castanospermine; 1,6,7,8-tetrahydroxyoctahydroindolizine alkaloid; structural determination.

Abstract—A new type of higher plant alkaloid, 1,6,7,8-tetrahydroxyoctahydroindolizine, designated castanospermine, has been isolated from the toxic seeds of the Australian legume *Castanospermum australe*.

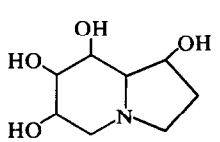
INTRODUCTION

Castanospermum australe, the only species of the genus *Castanospermum*, is a tall tree with smooth bark. The leaves are imparipinnate, 30–50 cm long, and the flowers are arranged in racemes usually on the old wood. The large coriaceous pods are 10–35 cm long and contain 3–5 large chestnut-like seeds. *C. australe* grows in rain forest and also along banks of streams in open country in north-eastern Australia.

The seeds of this species are frequently eaten by horses and cattle, and unripe seeds can cause severe gastro-intestinal irritation and sometimes death. The Australian aborigines use them as food after soaking them in water and roasting. The nature of the toxin is unknown [1].

While studying the distribution of non-protein amino acids in seeds of the Leguminosae we observed that extracts of the seeds of *C. australe* contained a basic compound which appeared as a brownish-yellow spot on 2-D chromatograms and ionophoresis papers after development with ninhydrin. The colour changed to purple after several days. The compound could not be identified from its R_f values or from its ionic mobility. The colour with ninhydrin suggested that the compound was probably a new non-protein amino acid or an alkaloid. Many alkaloids react with ninhydrin (giving a variety of colours) and numerous alkaloids, particularly quinolizidine alkaloids, have been identified in species of the tribe Sophoreae. The 'unknown' did not react, however, with either the Dragendorff or iodoplatinate reagents which are commonly used to detect alkaloids [2]. The compound was isolated and characterized as 1,6,7,8-tetrahydroxyoctahydroindolizine (**1**). The relative stereochemistry (**2**) was established by X-ray crystallography.

The isolated compound, designated castanospermine, is a octahydroindolizine alkaloid. Two other naturally occurring compounds are known to contain this ring system; these are δ-coniceine (**3**) from *Conium maculatum* [3–5] and the parasympathomimetic alkaloid slaframine (**4**) which occurs in the fungus *Rhizoctonia leguminicola* [6–8].



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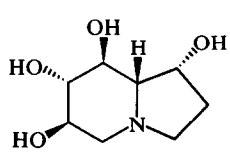

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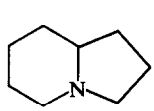

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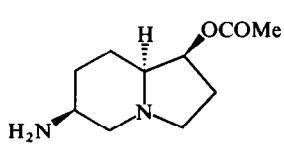

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RESULTS AND DISCUSSION

Elementary analysis and high resolution EIMS indicated that the molecular formula of the new compound was $C_8H_{15}NO_4$. The isolate resisted acid and alkaline hydrolysis and it was not reduced by hydrogen in the presence of platinum black. The UV spectrum showed only end absorption. The IR spectrum showed a prominent OH stretching frequency, but no evidence of a carboxyl group. No primary or secondary amino groups were detected, but periodic acid degraded the molecule indicating the presence of vicinal hydroxyl groups.

^{13}C NMR spectroscopy confirmed the presence of 8 aliphatic or alicyclic carbon atoms, all of which bore hydrogens (5 doublets and 3 triplets in the range δC 33.1–78.7); there was no indication of any unsaturated carbon atoms. The 360 MHz ^1H NMR spectrum of castanospermine is shown in Fig. 1. This spectrum, together with information from extensive double resonance experiments and the ^{13}C NMR data defines the complete connectivity of carbon and hydrogen atoms as shown in Fig. 2. Castanospermine formed a monomethiodide and a tetraacetate (360 MHz ^1H NMR acetate resonances at δ 1.97, 2.0, 2.0, 2.05 in $CDCl_3$). It was therefore a tetrahydroxy, tertiary amine. From its molecular formula and the absence of any other unsaturation it must be bicyclic with the nitrogen atom being the means of forming the bicycle since no further carbon–carbon bonds are allowed by ^1H NMR (Fig. 2) and all oxygens are taken up in OH groups. By reference to the chemical shifts of the protons in castanospermine the best interpretation of all the above data is structure **1**. Assignment of the ^{13}C NMR spectrum is shown in Fig. 3.

Fig. 4 is a computer-generated perspective drawing of the final X-ray model of 1(*R**),6(*R**), 7(*S**),8(*S**),9(*S**)-1,6,7,8-tetrahydroxyoctahydroindolizine. However, the X-ray diffraction experiment only defined the relative stereostructure, and the enantiomer shown is an arbitrary choice. The six-membered ring is in a chair conformation with all substituents in an equatorial orientation. The cyclopentane ring is in the C_2 conformation with the two-

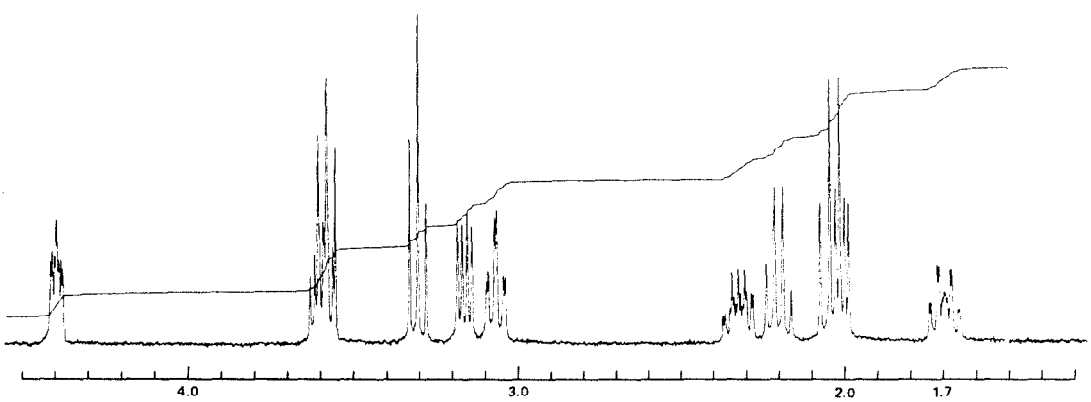

Fig. 1. 360 MHz ^1H NMR spectrum of castanospermine in D_2O.

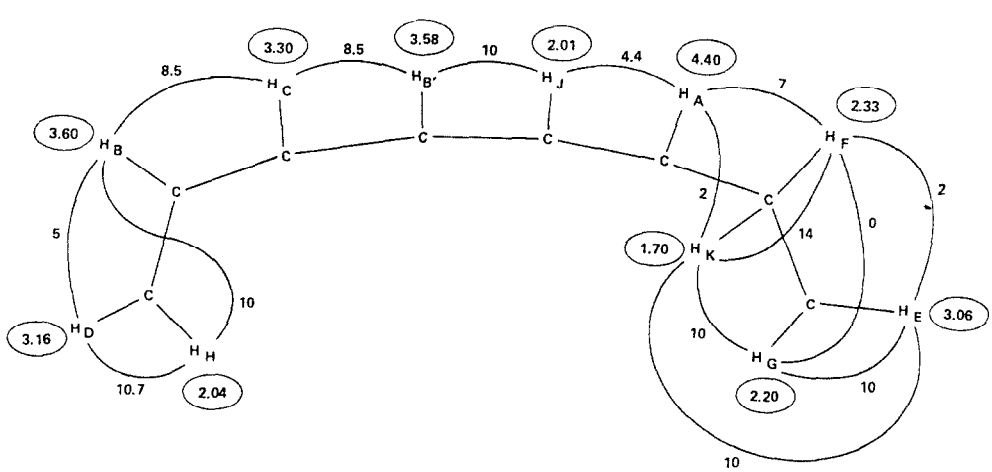

Fig. 2. Assignments of 360 MHz ^1H NMR spectrum of castanospermine. Encircled numbers are $y = \delta$ ppm from TMS (Fig. 1). H $\overset{x}{\frown}$ H $x = J_{H,H}$ Hz.

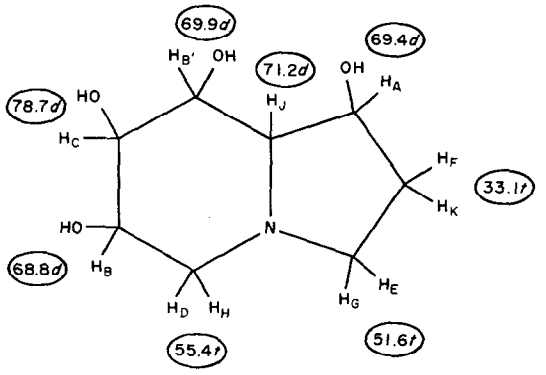

Fig. 3. Assignment of ^{13}C NMR spectrum of castano spermine. Based on selective proton irradiation at 90 MHz. Encircled numbers are $x = \delta$ C from TMS. D_2O solvent.

fold axis bisecting the N(4)–C(9) bond and containing C(2). There is a series of hydrogen bonds with ordered hydrogen positions: N(4)–HO(11)[2.74Å]–HO(10)-[2.84Å]–HO(13)[2.83Å]–HO(12)[2.67Å].

EXPERIMENTAL

Isolation of castanospermine. Finely ground immature seed (3 kg) of *C. australe* A. Cunn. (Sophoreae) was extracted with 75% EtOH (5 × 3 l.). After filtration the extract was concd under red. pres. to 0.5 l. and applied to a column (7 cm × 50 cm) of strongly acidic cation exchange resin (Dowex 50) in the H^+ form. After washing with H_2O the 'unknown' and the amino acids were displaced from the column with 2 M NH_3. The soln was concd under red. pres., diluted with H_2O and reconcd several times to remove NH_3. The soln was then applied to a column (7 cm × 40 cm) of Dowex 50 resin in the pyridinium form from which the acidic and neutral amino acids were removed by washing with 2 M pyridine. The 'unknown' and then arginine were displaced from the column with 2 M NH_3. The fractions containing the unknown by itself were combined and concd under red. pres. to a viscous brown syrup. After the syrup had been standing, large cubic crystals separated and these were recrystallized from aq. EtOH, yield 1.69 g, mp 212–215° decomp., $[\alpha]_D^{25}$ + 79.7° (*c* 0.93, H_2O). (Found: C, 50.77; H, 8.08; N, 7.30. $C_8H_{15}NO_4$ requires: C, 50.79; H, 7.94; N, 7.41%.) MS *m/e*: 189.1002 (calc. $C_8H_{15}NO_4$, 189.1001); 172.0972 (calc. $C_8H_{14}NO_3$, 172.974); 145.0737 (calc. $C_6H_{11}NO_3$, 145.0739); 86.0603 (calc. C_4H_8NO, 86.0606). UV no absorption >250 nm. ν_{max}^{KBr} cm^{-1}: 3100–3600 (br.). The 360 MHz ^1H NMR spectrum is shown in Fig. 1 and the values of ^{13}C NMR signals are given in Fig. 3.

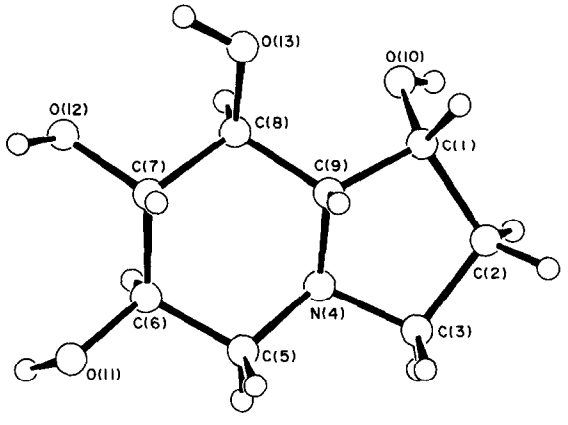

Fig. 4. Computer-generated perspective drawing of the final X-ray model of castanospermine.

2-D PC. Finely ground seed (200 mg) was shaken with 1 ml 70% EtOH for 24 hr. The suspension was allowed to settle and 0.01 ml of supernatant was chromatographed on Whatman No. 1 paper using the ascending method. Solvents used were (A) *n*-BuOH–HOAc–H_2O (12:3:5) followed by (B) PhOH–H_2O (4:1, w/v) in the presence of NH_3. Papers were developed with ninhydrin (0.2% w/v in 95% aq. Me_2CO). R_f values of castanospermine were 0.43 (A), 0.80 (B).

HV paper ionophoresis. Supernatant (0.01 ml) prepared as above was subjected to ionophoresis on Whatman 3 mm paper (70 V/cm for 30 min) in buffer solns of pH 1.9, 3.6 and 6.5 [9]. Ionic mobilities of castanospermine were 0.8_{ARG} (pH 1.9) and 0.3_{ARG} (pH 3.6 and 6.5).

^1H NMR. 360 MHz ^1H NMR spectra were obtained using a spectral width of 2 kHz, 16K data points, pulse width of 3 μsec (40° angle) and a 2-sec relaxation delay between acquisitions. The solvent was D_2O. Chemical shifts are computer-referenced to external TMS (HOD as 4.8 ppm).

^{13}C NMR. 22.5 MHz ^{13}C NMR spectra were obtained using a spectral width of 6 kHz 8K data points, pulse width 13 μsec (20° angle). Solvent D_2O using dioxan int. ref. at 66.5 ppm relative to TMS.

Synthesis of castanospermine methiodide. Castanospermine (2.7 mg) was suspended in dioxan (1 ml). Pyridine (0.1 ml) and Ac_2O (0.1 ml) were added and the mixture stirred at room temp. for 40 hr. The reaction mixture was evaporated to an oil under red. pres. The oil was recrystallized from H_2O giving 3.5 mg of derivative, mp 110–112°.

Synthesis of castospermine methiodide. Castanospermine (2.6 mg) was dissolved in EtOH (2 ml), iodomethane (0.06 ml) added and the mixture refluxed for 2 hr. The reaction mixture was evaporated to an oil (3.6 mg) under red. pres. The oil (which did not crystallize) was used directly for ^1H NMR studies.

X-Ray crystallography. Preliminary X-ray photographs of single crystals of castanospermine displayed monoclinic symmetry. Accurate lattice parameters, determined by a least-squares fit of 15 moderate, diffractometer measured 2θ-values, were **a** = 9.451(1), **b** = 8.182(1), **c** = 6.511(1) and β = 116.19(1)°. The presence of chirality, systematic extinctions and density considerations were uniquely accommodated by space group $P2_1$ with one molecule of $C_8H_{15}NO_4$ forming the asymmetric unit. All unique diffraction maxima with $2\theta \leq 114°$ were collected on a computer-controlled 4-circle diffractometer using a variable speed, 1° ω-scan and graphite monochromated Cu$K\bar{\alpha}$ radiation (1.64178 Å). After correction for Lorentz, polarization and background effects, 658 (99%) of the reflections were considered observed ($|F_o| \geqslant 3\sigma(F_o)$).

An initial phasing model was achieved by standard direct methods employing MULTAN [10, 11]. An *E*-synthesis calculated from the most favourable soln revealed the entire nonhydrogen structure. After partial refinement, a ΔF-synthesis using ORFLS [12] revealed the hydrogen atoms. Full-matrix least-squares refinements with anisotropic nonhydrogen atoms and isotropic hydrogens have currently converged to a standard crystallographic residual of 0.049 for the observed data. A final ΔF-synthesis showed no anomalously high electron density and there were no abnormally short intermolecular contacts. Tables of fractional coordinates, temperature factors, bond distances and bond angles have been deposited with the Cambridge

Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW and are also available from one of the authors (J.C.). Errors were calculated using ORFFE [13] and the crystallographic illustration used ORTEP [14].

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