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AUSTRALINE, A NOVEL PYRROLIZIDINE ALKALOID GLUCOSIDASE INHIBITOR FROM *CASTANOSPERMUM AUSTRALE*

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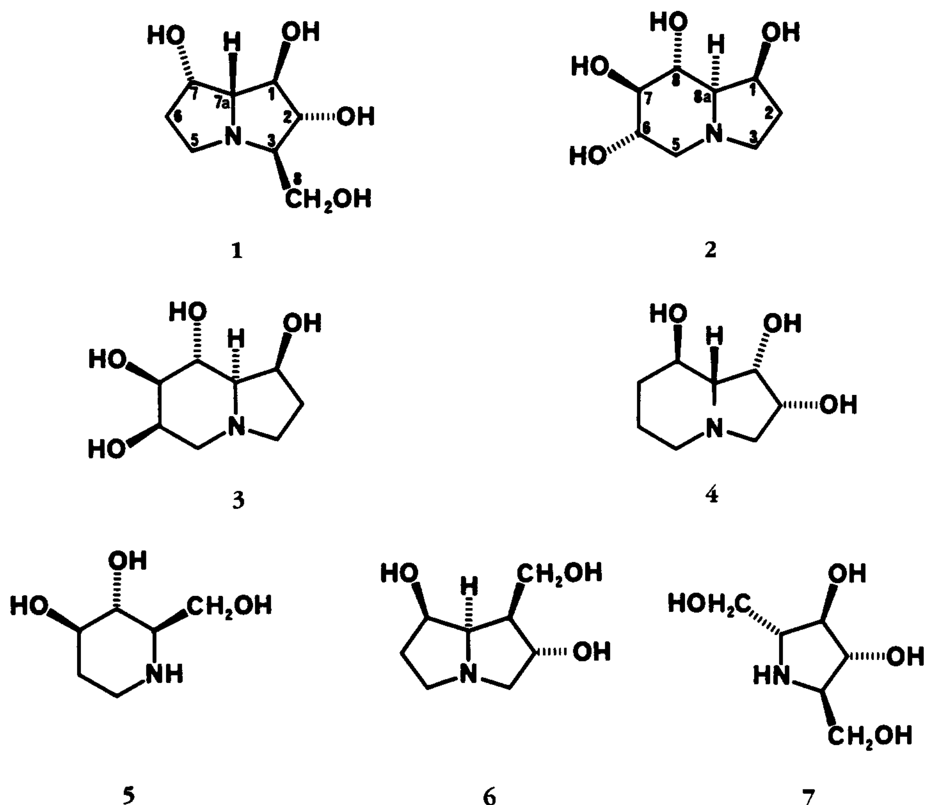
ABSTRACT.—Australine [**1**], a tetrahydroxy pyrrolizidine alkaloid with a unique substitution pattern, has been isolated from seeds of *Castanospermum australe* and shown to be a potent and specific inhibitor of amyloglucosidase. The structure was established by a combination of spectroscopic and chemical techniques and confirmed by X-ray crystallography. Australine is the first pyrrolizidine alkaloid to be identified as a glycosidase inhibitor and is the third glucosidase inhibitor, together with the indolizidine alkaloids castanospermine [**2**] and 6-*epi*-castanospermine [**3**], to be isolated from *C. australe*. In addition to these bicyclic alkaloids, the piperidine alkaloid fagomine [**5**], a non-inhibitor, has been isolated from the seeds.

Castanospermum australe A. Cunn. (Leguminosae), the Moreton Bay chestnut or black bean and the monotypic species of the genus *Castanospermum* native to northeastern Australia, has been introduced into the Indian subcontinent, South Africa, and mild climate areas of North America as an ornamental tree (1). Castanospermine [**2**], the major alkaloidal constituent of the toxic, chestnut-like seeds is a tetrahydroxyindolizidine alkaloid (2) having potent α- and β-glucosidase inhibitory activity (3,4). The consequent modification of glycoprotein processing in cells due to inhibition of glucosidase I (5) has stimulated considerable interest in the biological effects of castanospermine on a variety of organisms. The alkaloid is an intensely active feeding deterrent and toxin to certain insects (6,7) and adversely affects root length growth in a number of dicotyledonous plants (8). It has been shown to alter glycogen metabolism and distribution (9) and to block the hyperglycemic response to carbohydrates (10) in rats. In addition, castanospermine inhibits replication of the human immunodeficiency virus (HIV) (11) and other retroviruses (12) and reduces tumor growth in mice (13). Furthermore, the structurally related trihydroxyindolizidine alkaloid, swainsonine [**4**], the toxic constituent of locoweeds (14) and Australian *Swainsona* species (15), is a powerful inhibitor of α-mannosidase and exhibits anti-metastatic immunomodulation towards melanoma cells in mice (16). Such examples indicate that polyhydroxyindolizidine alkaloids and structurally related analogues may represent a general class of alkaloids capable of profoundly influencing diverse biological processes due to their glycosidase inhibitory activity (17) and have stimulated efforts to isolate or synthesize additional compounds in order to delineate structure-activity relationships.

Tlc of the mother liquors remaining after crystallization of castanospermine from extracts of *C. australe* seeds had indicated the presence of a number of other alkaloidal constituents. Separation by preparative centrifugal tlc yielded 6-*epi*-castanospermine [**3**], an inhibitor of α-glucosidase (18). In the present communication we report the isolation of fagomine [**5**], a known piperidine alkaloid, and a potent, specific α-glucosidase inhibitor, namely australine [**1**]. (Australine is named not only from the specific epithet of the plant species in which it occurs but also in recognition of the bicentennial of Australia, the country of origin of *C. australe*.) The structure of australine was established by application of one- and two-dimensional high-field nmr techniques and ms as a tetrahydroxypyrrolizidine alkaloid, and the stereochemistry of the substituents was confirmed by single crystal X-ray crystallography. This is the first report of a pyrrolizidine alkaloid with glycosidase inhibitory properties and represents a novel structural type within the class.



RESULTS AND DISCUSSION

Tlc of the *C. australe* crystallization mother liquors showed a number of components that were less mobile than castanospermine. One of these constituents gave an intense yellow spot with Ac_2O/Ehrlich's spray reagent and an orange spot with Dragendorff's reagent. Separation by preparative centrifugal tlc gave a crystalline solid, the melting point, optical rotation, ^{1}H-nmr and ms data of which were consistent with those of fagomine [**5**] (1,2,5-trideoxy-1,5-imino-D-arabino-hexitol), a piperidine alkaloid first isolated from buckwheat seeds (*Fagopyrum esculentum* Moench) (19). The ^{13}C nmr of fagomine was measured and assignments confirmed by a 2D heteronuclear ^{1}H-^{13}C shift correlation experiment. Although fagomine has not previously been reported to occur in Leguminosae, the 4-*O*-(β-D-glucopyranosyl)-derivative has been isolated from seeds of the legume *Xanthocercis zambesiaca* Baill. (20). No significant glycosidase inhibitory activity was found when fagomine was tested against commercially available α- and β-glucosidase, galactosidase, and mannosidase, in accord with previous results (20).

Australine [**1**] was isolated by repeated preparative centrifugal tlc as a colorless oil that was crystallized from Me_2CO with some difficulty as small, dextrorotatory prisms, mp 148–149°. The molecular formula was determined by hrms to be $C_8H_{15}NO_4$, isomeric with castanospermine, and the alkaloid formed a tetraacetate derivative under mild acetylation conditions indicating the presence of four primary or secondary hydroxyl groups. However, on tlc it gave a weak blue-gray spot with Ac_2O/Ehrlich's spray reagent rather than the intense purple spot characteristic of polyhydroxyindolizidine alkaloids. The latter color is produced by dehydration and rearrangement to give a pyrrole ring system which undergoes condensation with the 4-dimethylamino-

benzaldehyde at the position α to the nitrogen atom (21). The pyrrole ring generated from australine must, therefore, bear a substituent blocking the α position. Moreover, the eims showed no ion corresponding to the six-membered ring fragment, resulting from cleavage of bonds β to the nitrogen atom in the pyrrolidine ring moiety (17), typical for castanospermine and other indolizidine alkaloids. Instead the base peak occurred at *m/z* 158 $[M-31]^+$, indicating loss of an exocyclic -CH_2OH group. The fundamental ring system must, therefore, be of the pyrrolizidine type. Other major fragments observed in the mass spectrum were typical for this class of compound, resulting from cleavage of either of the five-membered rings at bonds β to the nitrogen atom. The masses of these ions were such that a single -OH group had to reside on one ring while the remaining two -OH groups and the -CH_2OH moiety fully substituted the alternate ring. In all saturated necine bases previously isolated, such as rosmarinecine [**6**], which bear the -CH_2OH group at the 1-position, the major mass spectral fragments result from cleavage of the ring system. In contrast, the major fragment from australine is a consequence of the loss of -CH_2OH, indicating that this substituent must be β to the nitrogen atom at the 3 position.

Conclusive evidence regarding the presence of a pyrrolizidine ring system and the position of substituents was obtained from the ^{13}C- and ^1H-nmr spectra, determined in D_2O. Whereas the ^{13}C spectrum of australine showed the presence of five methine and three methylene signals, as in castanospermine [**2**] and 6-*epi*-castanospermine [**3**], distinct chemical shift differences were immediately apparent. Thus, although two of the methylene signals (δ 38.1 and δ 54.9) corresponded closely to those in the five-membered ring of the latter alkaloids, the third signal at δ 65.6 occurred significantly downfield from those observed for methylene groups at the 5 position of castanospermine (δ 54.3) and 6-*epi*-castanospermine (δ 54.6). However, its value corresponded well with that observed for the symmetrically disposed -CH_2OH groups (δ 63.9) in dihydroxymethyl-dihydroxypyrrolidine (DMDP) [**7**] measured under identical conditions and, thus, confirmed the occurrence of such a substituent in australine.

The high resolution ^1H-nmr spectrum also demonstrated notable variations in chemical shifts of the well-separated groups of signals in comparison to those of the tetrahydroxyindolizidine alkaloids. In particular, while castanospermine and its 6-epimer show only a single signal below δ 4.0, corresponding to the proton at the hydroxylated 1 position, australine shows two signals at δ 4.58 and 4.41 together with a third at δ 3.92. In this respect it is comparable to swainsonine [**4**] which has -OH groups located at the 1 and 2 positions of the five-membered ring and exhibits two signals at δ 4.43 and 4.33, respectively, with a third at δ 3.88, all of which are due to protons on hydroxylated positions β to the nitrogen atom. In the high-field region at δ 1.5–2.5, australine showed only a two-proton multiplet, centered at δ 2.03, whereas castanospermine has five signals corresponding to the H-3β, H-5β, and bridgehead H-8a protons, which are essentially *trans*-diaxial to the nitrogen lone pair (22), together with both H-2 protons. However, the existence of only a single signal at lower field (δ 3.18) and two overlapping signals at δ 2.93 and 3.03 in australine indicated that the latter must be due to the 5α and 3α protons, which are *trans*-diaxial to the nitrogen lone pair. The differences in the ^1H spectra, which are much more apparent than those noted in the ^{13}C spectra, confirm the fundamental distinction between castanospermine and australine, namely the *cis*-fusion of the bicyclic ring system required for a pyrrolizidine alkaloid (22), rather than the energetically favored *trans*-fusion of the indolizidine alkaloids.

Extensive decoupling experiments established the connectivity and substitution pattern of australine as shown in structure **1** and yielded the requisite coupling constants. Of particular significance was irradiation of the lowest field signal at δ 4.58 that resulted in collapse of the high-field multiplet at δ 2.03, which must correspond to H-

6α and H-6β, and also the double doublet at δ 3.46, thus identifying the latter signal as the bridgehead H-7a. This resonance also collapsed upon irradiation of the low-field signal at δ 4.41, which must be due to H-1, since the only other signal affected was the double doublet at δ 3.92 (H-2). Irradiation of the multiplet at δ 3.03 (H-3) influenced not only the H-2 quartet but also the well-separated -CH_2OH proton signals at δ 3.64 and 3.81. The nonequivalence of these protons must be attributable either to restricted rotation (22) or to intramolecular hydrogen bonding of the hydroxyl group to the nitrogen lone pair. A 2D heteronuclear ^{1}H-^{13}C shift correlation experiment was used to confirm assignments of the ^{13}C-nmr chemical shifts; the results are shown in Figure 1. The signal for the -CH_2OH group occurred at δ 65.6 and the bridgehead C-7a at δ 68.9, as predicted from appropriate models (14). The four remaining methine carbon atoms, which are bound either to an oxygen or nitrogen atom, resonated in the narrow range of δ 72.9–77.3.

Determination of the coupling constants from the above experiments established the identical value of 5 Hz for $J_{1,7a}$, $J_{1,2}$, and $J_{7,7a}$ and a value of 9 Hz for $J_{2,3}$. The latter coupling constant is consistent with *trans* protons that exist in a near-diaxial situation

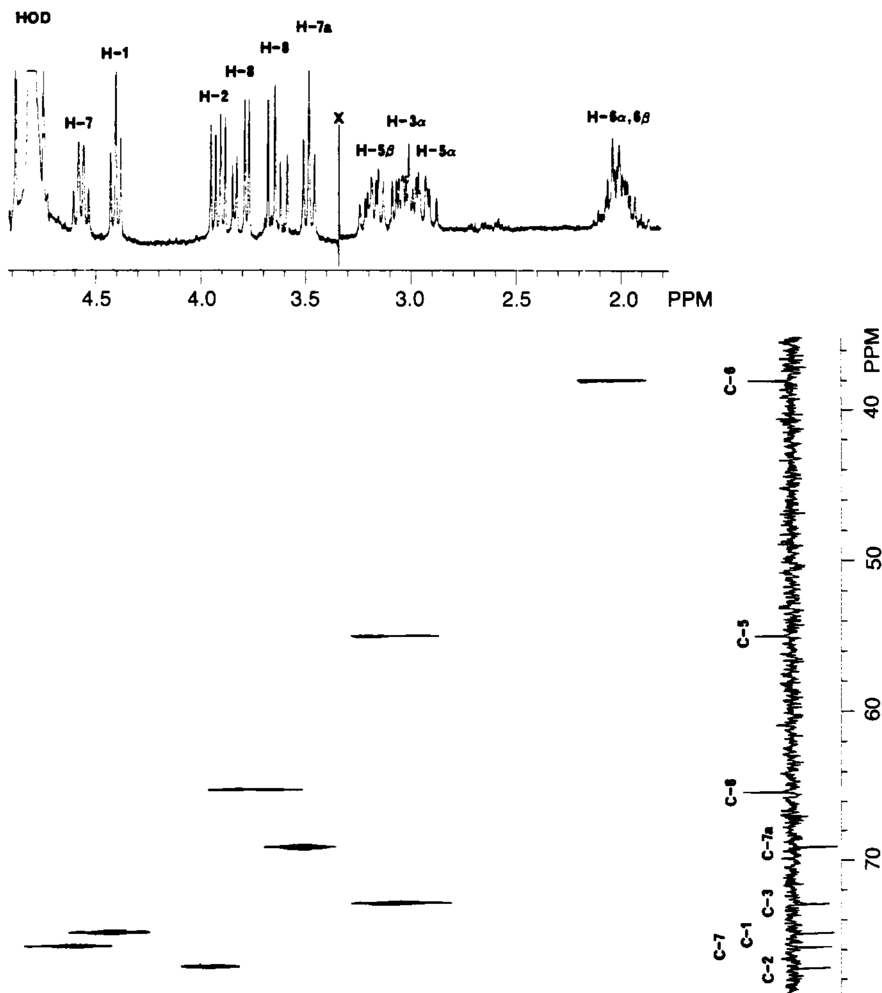


FIGURE 1. Two-dimensional ^{1}H-^{13}C nmr shift correlation spectrum of australine [1] in D_2O.

($\theta \approx 160°$) such as occur in croalbinecine (23) and rosmarinecine [**6**].[1] In australine, therefore, the 2-OH and the -CH_2OH groups must have a *trans* disposition to one another. In this event the magnetic nonequivalence of the -CH_2OH protons is unlikely to be due to restricted rotation. The 5 Hz coupling constants are unfortunately intermediate in magnitude between those reported for *cis* protons and for protons having a *trans* relationship in the above examples, and also in swainsonine [**4**] and its di- and triacetate derivatives (15).

Values observed for protons in a *cis* relationship ($\theta \approx 30°$) are generally in the range of 3–4 Hz, whereas those for *trans* protons are 8–10 Hz. Although the coupling constants of 5 Hz in australine are somewhat closer to those expected for *cis* substitution, chemical evidence argued against such a relationship, at least for the -OH groups in the 1 and 2 positions. Thus, treatment of the alkaloid with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid failed to yield an acetal derivative. Moreover, from examination of molecular models, the stereoisomer in which the -OH groups at the 1 and 7 positions bear a *cisoid* relationship to one another and are in close proximity might also have been expected to yield a six-membered ring acetal derivative. These results are, therefore, most consistent with structure **1** for australine in which the 1-OH group is *trans* to both of those at the 2 and 7 positions.

In view of the uncertainty of the stereochemical structure of australine based upon the nmr data, due partly to the known sensitivity of J values to slight variations in θ in five-membered ring systems, together with the potential for *exo*- or *endo*-flipping in saturated pyrrolizidine alkaloids (22), it was deemed essential to ascertain the stereochemistry by an unequivocal method. Crystals sufficiently large for X-ray crystallographic analysis were obtained, with some difficulty, by slow crystallization from Me_2CO and the structure, stereochemistry, and absolute configuration of australine thereby established as (1*R*,2*R*,3*R*,7*S*,7a*R*)-3-hydroxymethyl-1,2,7-trihydroxypyrrolizidine [**1**]. The molecular conformation is illustrated as a perspective drawing in Figure 2. The crystal structure consists of two crystallographically independent

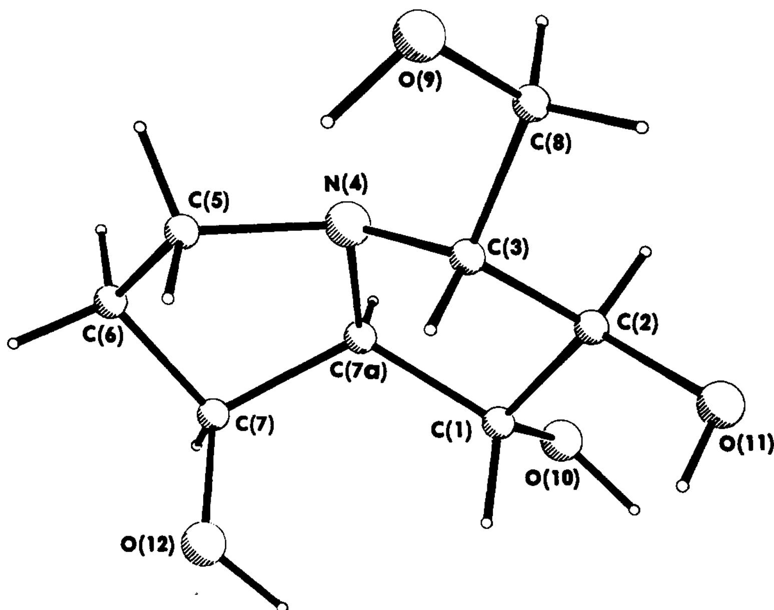


FIGURE 2. Perspective view of australine [**1**] with crystallographic numbering scheme.

[1]R.J. Molyneux and M. Benson, unpublished observations.

molecules within each asymmetric unit. Except for minor differences in the values of analogous bond lengths and bond angles, both molecules possess similar structural features. The two five-membered rings are symmetrically *endo*-buckled with C-2 and C-6 being 0.6 Å above the mean plane of the other four atoms of their respective rings. The *endo*-buckling presumably reduces non-bonded interaction between H-3α and H-5α and between H-1 and the -OH group at the 7 position (22). The molecule is folded along the nitrogen-bridgehead carbon bond, resulting in a dihedral angle of approximately 54° between the two ring planes. The three hydroxyl groups are in a *transoid* disposition to one another, and the orientation of the -CH_2OH substituent is noteworthy. It assumes a position with the hydroxyl group directed towards the nitrogen lone pair, enhancing the formation of an intramolecular hydrogen bond [O(9) . . . N(4) = 3.1 Å] and concomitant five-membered chelate ring, thus accounting for the non-equivalence of the -CH_2OH protons in the nmr spectrum.

The molecules in the crystal structure mutually interact with adjacent molecules through an intricate arrangement of intermolecular hydrogen bonds formed by the nitrogen and oxygen atoms of adjacent unit molecules. (X-ray crystallographic data will be published in detail elsewhere.) The short intermolecular contacts between oxygen-oxygen and oxygen-nitrogen atoms are within the range of 2.68–2.89 Å. This unusual feature of the crystal structure, comprising two crystallographically independent molecules per asymmetric unit, is possibly due to the tendency of these molecules to form a considerable number of relatively strong hydrogen bonds between one another, enhancing a more compact and stable molecular packing in the crystal.

The co-occurrence of australine [**1**] with castanospermine [**2**] and 6-*epi*-castanospermine [**3**], which are known glycosidase inhibitors, suggested that the former should also be evaluated as an inhibitor of a number of readily available glycosidases. The trihydroxypyrrolizidine alkaloid rosmarinecine [**6**] had been tested earlier and found to lack activity. However, australine proved to be a potent and specific inhibitor of amyloglucosidase [an *exo*-1,4-α-glucosidase (K_i = 5.2 μM)] whereas castanospermine inhibited both α-glucosidase (K_i = 7.9 μM) and β-glucosidase. No significant inhibition of australine of β-glucosidase, α- and β-mannosidase, or α- and β-galactosidase was observed. Detailed studies of the inhibition of α-glucosidase and the effect of australine on glycoprotein processing will be published elsewhere.

Australine [**1**] is, therefore, not only the first tetrahydroxypyrrolizidine alkaloid to be isolated but also possesses a unique substitution pattern, bearing the hydroxymethyl group at the 3 position rather than the previously invariant 1 position. In addition it is the sole member of the pyrrolizidine class to be shown to be a glycosidase inhibitor. Previously identified classes of inhibitors have been limited to the polyhydroxyindolizidines (e.g., castanospermine), polyhydroxypyrrolidines (e.g., DMDP), and polyhydroxylpiperidines (e.g., nojirimycin). It is interesting to speculate whether polyhydroxyquinolizidine alkaloids, if they exist in nature or can be synthesized, might also prove to have glycosidase inhibitory properties. It has been postulated (17) that the sine qua non for polyhydroxy alkaloids to possess such activity is the requirement that at least three hydroxyl groups be located in a β position relative to the nitrogen atom. All known inhibitors conform to this requirement, including australine [**1**], whereas the non-inhibitors fagomine [**5**] and rosemarinecine [**6**] only have two hydroxyl groups so situated. Such a condition may provide a working model for the synthesis or selection of additional alkaloids for evaluation, specific inhibitory properties being dependent upon the relative stereochemistry of the hydroxyl groups. It should be noted, however, that while australine [**1**] and DMDP [**7**] have the same relative stereochemistry, the latter inhibits both α- and β-glucosidase whereas australine is a specific inhibitor of α-glucosidase.

The structure of australine [1] raises some intriguing questions as to its biogenetic origin. Its co-occurrence with castanospermine [2] in *C. australe* suggests that it may be formally regarded as an indolizidine alkaloid in which the highly hydroxylated six-membered ring has undergone ring contraction. (The structure of australine [1] is presented in the conventional manner for all pyrrolizidine alkaloids, namely with the most highly substituted ring on the right. Its relationship to castanospermine [2] can be visualized by 180° rotation about the C-7a, N axis.) More probable is biosynthetic elaboration from a polyhydroxypyrrolidine such as DMDP [7] by a two-carbon addition, possibly acetate-derived, followed by subsequent cyclization to give the alternate five-membered ring. Appropriate biosynthetic experiments will be required to resolve the biogenesis of australine.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The alkaloids were monitored for purity by tlc on 0.25-mm Si gel plates developed with $CHCl_3$-MeOH-NH_4OH-H_2O (70:26:2:2) and detected by spraying with Ac_2O followed by Ehrlich's reagent (21). Homogeneity was also checked by gc of the trimethylsilyl derivatives, prepared by treatment with MSTFA in pyridine, on a Hewlett-Packard 5830 instrument equipped with a flame-ionization detector, on-column injector, and a 30-m × 0.32-mm i.d. SE-30 fused silica column. Low resolution electron and NH_3 cims were obtained on a VG Micromass 7070 mass spectrometer; high resolution mass measurements were determined on the same instrument. Optical rotations were measured in a 1-dm cell on a Perkin-Elmer 241 automatic polarimeter. Nmr spectra were determined in D_2O on a Nicolet NTC 200FT spectrometer with software package at 200 MHz (^1H) and at 50.3 MHz (^{13}C) using 3-(trimethylsilyl)-1-propanesulfonic acid (TSP) as an internal standard. Multiplicities for ^{13}C signals were determined by application of the carbon attached proton test (CAPT) sequence. Preparative centrifugal tlc was performed on a Chromatotron Model 7924 (Harrison Research, Palo Alto, CA). Enzyme assay methods have been previously described (18). Melting points are uncorrected.

PLANT MATERIAL.—Seeds of *C. australe* were collected from the Huntington Botanical Gardens, San Marino, California in November 1983.

EXTRACTION AND FRACTIONATION.—Mature seeds were ground and extracted with MeOH in a Soxhlet apparatus and the alkaloidal fraction purified by ion-exchange chromatography on Dowex 50W-X8 (NH^+ form) (2). The eluate was repeatedly subjected to crystallization from MeOH until no additional castanospermine, mp 217–219°, could be isolated. Portions of crystallization mother liquors (200 mg) were fractionated by preparative centrifugal tlc on a 2 mm Si gel plate by sequential elution with $CHCl_3$-MeOH-NH_4OH-H_2O (70:26:2:2) (120 ml) and EtOH-NH_4OH (98:2) (200 ml). A total of 70 fractions (ca. 4.5 ml each) were collected. Fractions showing similar tlc profiles were pooled and concentrated. Residual castanospermine eluted in fractions 21–32 (70 mg).

ISOLATION AND CHARACTERIZATION OF FAGOMINE [5].—Combined fractions 31–44 (22 mg) gave a discrete bright yellow spot on tlc (R_f = 0.12) which was slightly less mobile than castanospermine (R_f = 0.14). Gc analysis of the trimethylsilyl derivative gave a single peak with a retention time (Rt) of 12.07 min. Castanospermine has Rt 19.22 min. The colorless oil slowly crystallized and was recrystallized from MeOH/EtOH as white cubes, mp 184–185° [lit. (19) mp for fagomine 186–188°]. The ^1H-nmr and mass spectra were completely in accord with data previously reported for fagomine (19); ^{13}C nmr (50.3 MHz, D_2O) δ 75.9 (C-3), 75.8 (C-4), 64.2 (C-6), 63.6 (C-5), 45.3 (C-1), 35.3 (C-2); $[\alpha]^{24}$ λ (c = 0.51, MeOH) +24.4° (589), +25.5° (578), +28.9° (546), +48.5° (436), +73.9° (365) {lit. (20) $[\alpha]^{20}D$ = 24.7° (c = 0.4, H_2O)}. Fagomine showed no inhibitory activity when tested against a number of generally available glycosidases, as previously reported (20).

ISOLATION AND CHARACTERIZATION OF AUSTRALINE [1].—Evaporation of combined fractions 45–65 gave a clear, colorless oil (61 mg) which showed a blue-gray spot on tlc (R_f = 0.08). Gc of the trimethylsilyl derivative gave a single peak with Rt 17.07 min. Crystallization from Me_2CO afforded australine [1] as colorless prisms, mp 148–149°; $[\alpha]^{26}$ λ (c = 2.09, MeOH) +19.3° (589), +19.9° (578), +22.1° (546), +32.8° (436), +42.3° (365); ^1H nmr (200 MHz, D_2O) δ 4.58 (1H, dd, $J_{6\alpha,7}$ = 9 Hz, $J_{7,7a}$ = 5 Hz, H-7), 4.41 (1H, dd, $J_{1,2}$ = $J_{1,7a}$ = 5 Hz, H-1), 3.92 (1H, dd, $J_{1,2}$ = 5 Hz, $J_{2,3}$ = 9 Hz, H-2), 3.81 (1H, dd, $J_{3,8}$ = 4 Hz, J_{gem} = −12 Hz, H-8), 3.64 (1H, dd, $J_{3,8}$ = 6.5 Hz, J_{gem} = −12 Hz, H-8), 3.46 (1H, dd, $J_{1,7a}$ = $J_{7,7a}$ = 5 Hz, H-7a), 3.18 (1H, m, H-5β), 3.03 (1H, m, H-3α), 2.93 (1H, m, H-5α), 2.02 (2H, m, H-2α,2β); ^{13}C nmr (50.3 MHz, D_2O) δ 77.3 (C-2), 75.8 (C-7), 74.9 (C-1), 72.9 (C-3), 68.9 (C-7a), 65.6 (C-8), 54.9 (C-5), 38.1 (C-6); ^1H-^{13}C 2D shift correlation nmr (D_2O) see Figure 1;

eims *m/z* $[M]^+$ 189 (5), 159 (16), $[M-CH_2OH]^+$ 158 (100), 129 (10), 128 (14), 114 (11), 112 (43), 98 (8), 86 (38), 70 (22); cims (NH_3) *m/z* $[MH]^+$ 190 (100), $[MH-H_2O]^+$ 172 (6), 158 (40); hrms mass measurement $[M]^+$ *m/z* 189.0998 (calcd for $C_8H_{15}NO_4$, 189.1001).

PREPARATION OF AUSTRALINE TETRAACETATE.—Australine (10 mg) in dry pyridine (0.3 ml) was treated with Ac_2O (0.5 ml) and kept at room temperature for 20 h. The clear solution was poured into H_2O (20 ml), the aqueous mixture extracted with $CHCl_3$ (3 × 10 ml), and the organic layer dried over anhydrous $MgSO_4$, filtered, and evaporated to give a colorless oil (15 mg): ^1H nmr (200 MHz, $CDCl_3$) δ 5.47 (1H, dd), 5.27 (2H, m), 4.17 (1H, dd), 4.04 (1H, dd), 3.54 (1H, q), 3.22 (1H, m), 3.13 (1H, m), 2.71 (1H, m), 2.15–2.00 (2H, m), 2.13 (3H, s, -OAc), 2.09 (3H, s, -OAc), 2.08 (3H, s, -OAc), 2.04 (3H, s, -OAc); ^{13}C nmr (50.3 MHz, $CDCl_3$) δ 170.7 (-OCOMe), 170.5 (-OCOMe), 170.0 (-OCOMe), 169.8 (-OCOMe), 77.9 (C-6), 73.8 (C-1), 73.4 (C-7), 69.5 (C-5), 66.8 (C-7a), 64.4 (C-8), 52.1 (C-3), 33.9 (C-2), 21.1 ($-OCOCH_3$), 20.84 (2 × $-OCOCH_3$), 20.76 ($-OCOCH_3$); eims *m/z* $[M]^+$ 357 (0.8), $[M-Ac]^+$ 314 (1.3), 297 (22), $[M-CH_2OAc]^+$ 284 (40), 237 (9), 182 (21), 178 (100), 164 (54), 122 (50), 43 (98); cims (NH_3) *m/z* $[MH]^+$ 358 (100), 316 (3), 297 (3), 284 (3), 240 (4), 236 (8), 178 (16), 164 (6), 136 (6), 118 (7); hrms mass measurement $[M]^+$ *m/z* 357.1431 (calcd for $C_{16}H_{23}NO_8$, 357.1423).

X-RAY STRUCTURE DETERMINATION.—A single crystal of australine, monoclinic space group, $P2_1$, a = 8.132(2), b = 9.337(2), c = 12.219(3) Å, β = 106.61(2)° was used. Intensity data was collected on a Nicolet R3 diffractometer with graphite monochromatized Cu-K_α radiation (λ = 1.5418 Å) by the θ–2θ scan technique at room temperature. The crystal structure was solved by direct methods. The final least squares structure refinement converged at $R = 0.050$ and R (weighted) = 0.063. The absolute configuration was determined by comparison of R values for the two enantiomeric structures.

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