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1-EPIAUSTRALINE, A NEW PYRROLIZIDINE ALKALOID FROM *CASTANOSPERMUM AUSTRALE*

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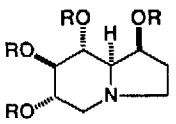
A new pyrrolizidine alkaloid [**6a**] has been isolated from the legume *Castanospermum australe* A. Cunn. (Leguminosae) and identified as (1*S*,2*R*,3*R*,7*S*,7a*R*)-3-hydroxymethyl-1,2,7-trihydroxypyrrolizidine on the basis of ^{1}H NMR nuclear Overhauser effects and other spectroscopic studies.

The Australian leguminous plant *Castanospermum australe* A. Cunn. (Leguminosae) is proving to be a rich source of novel polyhydroxylated alkaloids which are inhibitors of many glycosidases.[1-3] The dominant alkaloid is the indolizidine castanospermine[4] [**1a**]; it and other members of this group have become useful tools in the study of glycoprotein processing and show promise as antineoplastic and antiretroviral agents. Two pyrrolizidine alkaloids have been isolated from *C. australe*, australine[5] [**2a**] and 3-epiaustraline[6] [**3a**]; these are members of a new class of pyrrolizidines possessing carbon substituents at C-3 rather than at C-7. The first of the class, alexine [**4**], was isolated by Nash et al. from *Alexa leiopetala*,[7] another plant which produces castanospermine.[8] As the first step in a study of the biosynthesis of these indolizidines and pyrrolizidines, we are investigating the range of alkaloids present in *C. australe* in the hope of identifying constituents which might shed light on the biosynthetic pathway. The biosynthesis by *Rhizoctonia leguminicola* and *Astragalus oxyphysus* of a related trihydroxylated indolizidine alkaloid, the α-mannosidase inhibitor swainsonine [**5**], proceeds from pipecolic acid through mono- and dihydroxyindolizidine species.[9,10] We had hoped to find mono-, di-, or trihydroxylated indolizidines or pyrrolizidines in *C. australe* also. Although this hope has not yet been realized, a new pyrrolizidine alkaloid was isolated, the structure of which [**6a**] is the subject of this report.

Dried leaves from a young plant were ground and extracted with 95% ethanol. The extract was dried, dissolved in water, delipidated with methylene chloride and passed through a Dowex 50 cation-exchange column. Elution with ammonia yielded an alkaloid-rich fraction which was treated with acetic anhydride to acylate alcohols and any primary or secondary amines that might be present. The alkaloids were fractionated on silica gel as previously described.[10] Of



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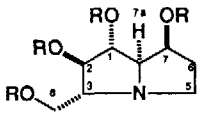
b R = Ac



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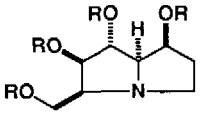
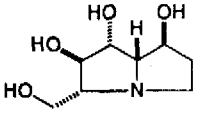


3a R = H
b R = Ac



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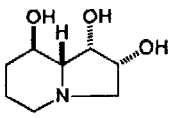

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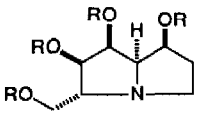
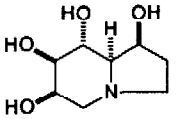


6a R = H
b R = Ac



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the tertiary amine esters which were found, castanospermine tetraacetate [**1b**] eluted first followed by the tetraacetate of 6-epicastanospermine [**7**].[11] The succeeding fractions contained a mixture of alkaloids which were further purified by either preparative tlc or centrifugal tlc on silica gel. The most mobile component was identified as australine tetraacetate [**2b**] by comparison of its ^{1}H NMR spectrum with that reported by Molyneux et al.[5] The least mobile was recognized as 3-epiaustraline tetraacetate [**3b**] on the basis of the downfield shift of H-3 and the smaller coupling constant between H-2 and H-3 when compared to **2b** (5.4 Hz vs 8.8 Hz). Hydrolysis of **3b** with K_2CO_3 in methanol gave **3a** for which the structural assignment was confirmed by comparison of the ^{1}H nmr spectrum to that reported by Nash et al.[6] Tetraacetate **6b**[12] eluted between **2b** and **3b**. Treatment of **6b** with K_2CO_3/MeOH gave tetraol **6a**;[13] **6a** could also be isolated from the seeds of *C. australe*.

The molecular weight (357) of **6b** together with the presence of four acetyl groups (as seen in the ^{1}H nmr spectrum) indicated that **6a** is an isomer of previously isolated *Castanospermum* alkaloids. The ^{1}H nmr assignments for **6b** (see Table) were made by COSY and specific decoupling experiments. The presence of geminal -CH_2O- protons at δ 4.17 and 4.03 (J_{gem} = 11.4 Hz) and a fragment ion at M-73 in the mass spectrum (loss of CH_2OAc) suggested that the unknown compound belonged to the new class of pyrrolizidine alkaloids which have a hydroxymethyl group α to the nitrogen. Proton relationships deduced from the COSY spectrum indicated that the new alkaloid must have two hydroxyl groups and the hydroxymethyl group on one ring at positions 1, 2, and 3, respectively, and the fourth hydroxyl group at position 7 on the other ring. Hence the alkaloid has the same skeleton as the previously identified pyrrolizidines **1a**, **3a**, and **4** and must differ only in stereochemistry. Attempts to crystallize the tetraacetate or the free base (after removal of the acetyl groups) were unsuccessful which precluded structure determination by X-ray diffraction.

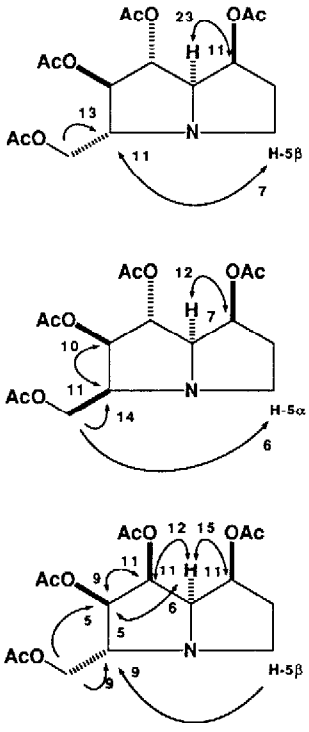
Chemical shifts and coupling constants (see Table) were of limited value for assigning stereochemistry because of uncertainty as to the conformation(s) of the fused five-member ring systems.[14,15] However, comparison of **6b** to **2b** and **3b** by nOe difference spectroscopy allowed the relative stereochemistry to be assigned. Key nOe's are shown in the Figure. Australine tetraacetate [**2b**] showed a strong nOe from H-7a to H-7. Irradiation of H-7 gave a strong reciprocal nOe; however, H-7 and H-1 are nearly isochronous leading to some ambiguity. A strong nOe was seen from H-8 and H-8' to H-3 and in the other two alkaloids as well. Most significantly, nOe's were observed between H-3 and *one* of the protons on C-5. The proton affected is the more upfield of the two. It can tentatively be assigned as the β proton on the basis of earlier assignments in pyrrolizidine alkaloids[14,15] and also of the 'syn upfield rule' of Anteunis and Danneels;[16] this empirical rule has been used for assignment of NMR signals of 5-membered rings where application of Karplus relationships is difficult due to uncertainties concerning conformation and dynamics. Certain

Chemical Shifts And Selected Coupling Constants[a]

Proton	2b	3b	6b
H-1	5.25	5.17	5.42
H-2	5.44	5.31	5.08
H-3	3.05	3.54	3.31
H-5α	3.19	3.07	3.17
H-5β	2.68	2.72	2.92
H-6	2.08	2.20	2.00
H-6'	2.00	2.00	2.00
H-7	5.25	5.32	5.29
H-7a	3.52	3.43	3.85
H-8	4.15	4.30	4.17
H-8'	4.03	4.27	4.03
Acetate	2.10	2.09	2.08
Acetate	2.08	2.07	2.06
Acetate	2.07	2.06	2.00
Acetate	2.02	2.03	1.98
$J_{1,2}$	7.2 Hz	2.7 Hz	4.2 Hz
$J_{1,7a}$	6.3	5.3	3.8
$J_{2,3}$	8.8	5.4	9.2
$J_{7,7a}$	4.3	~5.5	7.3
$J_{3,8}$	4.1	6.2	4.1
$J_{3,8'}$	5.5	7.1	5.7

[a] Solutions in $CDCl_3$. Relative configurations of H-6 and H-6' and of H-8 and H-8' have not been assigned.

NOE's in Pyrrolizidine Alkaloids



nOe's are absent. NOe's are not observed between H-1 and H-2 or between H-2 and H-3, consistent with the fact that the 2-acetoxy group is on the opposite face from the substituents at C-1 and C-3.

A similar study of nOe's for 3-epiaustraline tetraacetate [**3b**] revealed reciprocal nOe's between H-7 and H-7a, and lack of nOe's between H-7a and H-1 or between H-1 and H-2. However, reciprocal nOe's were seen between H-2 and H-3, consistent with the 1-acetoxy group being α and the 2- and 7-acetoxy groups being β as is the CH_2OAc group. An nOe was seen from one of the C-8 protons to one of the C-5 protons. The C-5 proton involved is the more downfield one which must tentatively be assigned as H-5α. While it is possible that a violation of the 'syn upfield rule' is occurring due to unusual conformations created by the β location of the CH_2OAc group, it seems more likely that there is deformation of the rings in **3b**, for example, by the left ring being *exo*-buckled while the right ring is *endo*, such that the CH_2OAc group lies closer to H-5α than to H-5β. In spite of this uncertainty, the nOe supports the structure of **3b** since an α-linked substituent at C-3 would be *exo* and would not be capable of giving an nOe to any proton in the other ring.

The downfield shifts of H-1 and H-7a and smaller vicinal coupling constant between them ($J_{1,7a}$ = 3.8 Hz) in **6b** suggested that it differed from **2b** and **3b** in stereochemistry at or near the bridgehead. Compound **6b** showed good nOe's between H-7 to H-7a, indicating that these protons are *cis* as they are in **2b** and **3b**. In contrast to **2b** and **3b**, H-7a of **6b** also showed a strong nOe to H-1 indicating a *cis* orientation of these protons. A strong nOe from H-1 to H-2 indicated that H-2 had the same orientation as in **2b** and **3b**. The *cis* orientation of the hydroxyl groups at C-1 and C-2 in **6a** was confirmed by the strong retardation of **6a** when chromatographed on borate-impregnated silica gel. H-2 showed nOe's to both of the C-8 protons but not to H-3 (in contrast to **3b**). A trans diaxial arrangement of H-2 and H-3 is also supported by a 9.2 Hz coupling constant observed between them. In **6b** as in **2b**, an nOe was

observed from H-5β to one of the protons on C-8. The overall patterns provide strong evidence that the new alkaloid differs from australine only in the configuration at C-1. Inasmuch as all of the hitherto isolated bicyclic alkaloids from *C. australe* have the same (*R*) stereochemistry at the bridgehead carbon, we propose that the new alkaloid is (1*S*,2R,3*R*,7*S*,7a*R*)-3-hydroxymethyl-1,2,7-trihydroxypyrrolizidine (1-epiaustraline).[17]

1-Epiaustraline was tested for glycosidase inhibition and found to inhibit the α-glucosidase amyloglucosidase (50% inhibition at 2.6 x 10^{-5} M), although not quite as well as castanospermine and australine.[18] It also inhibits glucosidase I, β-glucosidase, and α-mannosidase at millimolar levels but fails to affect glucosidase II. Interestingly, it inhibited yeast α-glucosidase (50% inhibition at 2.7 x 10^{-4} M), an activity not demonstrated by other α-glucosidase inhibitors.

ACKNOWLEDGEMENTS Support by NIH ES-00267 (T.M.H.) and HL-17783 (A.D.E.) and by the Robert E. Welch Foundation (A.D.E.) is gratefully acknowledged. Low resolution mass spectra were obtained by Betty Fox and Brian Nobes, Department of Pharmacology, Vanderbilt University; high resolution measurements were performed by Roger England at the Western Regional Research Center.

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12. Dried leaves (937 g) yielded 4.5 mg of **6b** as a colorless oil; $[\alpha]_D^{26}$ -15° (c=1.22, MeOH); ^{13}C nmr ($CDCl_3$) δ 170.69, 170.31, 169.58, 169.35 ($\underline{C}H_3\underline{C}O$), 74.51 (C-7), 73.89 (C-2), 71.92 (C-1), 66.92 (C-3), 65.15 (C-8), 64.90 (C-7a), 52.51 (C-5), 31.04 (C-6), 21.03, 20.85 (x2), 20.52 ($\underline{C}H_3CO$); cims (CH_4) m/z $[MH]^+$ 358 (100), [MH-HOAc] 298 (38), 284 (29), 178 (69).
13. **6a**: an amorphous white solid; $[\alpha]_D^{22}$ +12° (c=1.17, H_2O); ^1H nmr (D_2O-DCl, pH 1.25) δ 4.59 (1H, H-1), 4.21 (1H, H-2), 4.77 (1H, H-7), 4.19 (1H, H-7a), 4.05 (1H, H-8), 3.96 (1H, H-8), 3.70 (1H, H-5), 3.66 (1H, H-3), 3.55 (1H, H-5'), 2.36 (1H, H-6'), 2.26 (1H, H-6); ^{13}C nmr (D_2O-DCl, pH 1.25) δ 75.11, 73.60, 72.69, 70.66, 66.62, 63.44, 52.59, 35.87; hrms mass measurement $[M]^+$ m/z 189.1012 (calcd for $C_8H_{15}NO_4$, 189.1001), $[M-CH_2OH]$ 158.0828 (calcd for $C_7H_{12}NO_3$, 158.0817).
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