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ISOLATION FROM CASTANOSPERMUM AUSTRALE AND X-RAY CRYSTAL STRUCTURE OF 3,8-DIEPIALEXINE, (1R,2R,3S,7S,8R)-3-HYDROXYMETHYL-1,2,7-TRIHYDROXYPYRROLIZIDINE [(2S,3R,4R,5S,6R)-2-HYDROXYMETHYL-1-AZABICYCLO[3.3.0]OCTAN-3,4,6-TRIOL]

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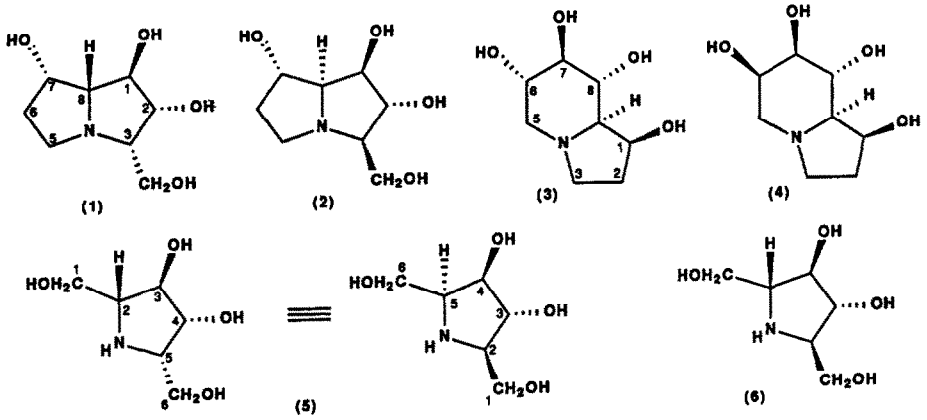
The isolation from Castanospermum australe and identification by X-ray crystal structure analysis of (1R,2R,3S,7S,8R)-3-hydroxymethyl-1,2,7-trihydroxypyrrolizidine [(2S,3R,4R,5S,6R)-2-hydroxymethyl-1-azabicyclo[3.3.0]octan-3,4,6-triol], the second example of a pyrrolizidine alkaloid with a carbon substituent at C-1, is described. A study of the inhibition of glycosidases by 3,8-diepialexine is reported.

This paper reports the isolation from seeds of the monotypic Australian rainforest and riverine tree Castanospermum australe of 3,8-diepialexine (1) [(1R,2R,3S,7S,8R)-3-hydroxymethyl-1,2,7-trihydroxypyrrolizidine.]. Although pyrrolizidine alkaloids with a carbon substituent at C-1 are common,[1] the first example of an alkaloid with a substituent at C-3, alexine (2), was isolated from Alexa leiopetala recently.[2] Two epimeric tetrahydroxylated octahydroindolizine alkaloids have been isolated from Castanospermum australe; castanospermine (3)[3] is an inhibitor of several α and β glucosidases[4] including glucosidase I of glycoprotein processing.[5] Castanospermine has been shown to inhibit experimental metastasis in mice,[6] and human immunodeficiency virus (AIDS) infectivity in vitro;[7] such compounds may have potential as antiretroviral agents.[8] 6-Epicastanospermine (4),[9,10] also isolated from Castanospermum australe,[11] is structurally related to deoxymannojirimycin in the same way that castanospermine (3) is related to deoxynojirimycin; however, (4) is a powerful inhibitor of amyloglucosidase (an exo-1,4-α-glucosidase) and does not inhibit either Jack bean α-mannosidase or almond β-glucosidase. Recently, castanospermine has been isolated from the dried pod of Alexa leiopetala and also tentatively identified in seven other species of the same genus.[12] Alexa spp. (Sophoreae, Leguminosae) are trees native to the wet lands of Guyana, Surinam, French Guiana, Venezuela and the Amazon basin; despite their geographical separation from Castanospermum australe, the common possession of castanospermine and the similarity of structure between 3,8-diepialexine and alexine suggest the two genera are closely related. A detailed taxonomic study will be reported elsewhere.[13]

Both 3,8-diepialexine and alexine are structurally related to 2,5-dideoxy-2,5-imino-D-glucitol (5); alexine (2) may formally be derived by linking C-1 by an ethano bridge to the nitrogen of (5), whereas 3,8-diepialexine (1) might be derived by linking C-6 with an ethano bridge to the nitrogen of (5). Although the iminoglucitol (5) has not been been isolated as a natural product, the epimeric 2,5-dideoxy-2,5-imino-D-mannitol, DMDP, (6) is an alkaloidal glucosidase inhibitor[14] which occurs in some spp. of Derris and Lonchocarpus (Leguminosae).[15]

Isolation. Finely ground freeze-dried seed (200 g) of Castanospermum australe A. Cunn, (Queensland Herbarium voucher no. BRI AQ426819-M. P. Hegarty) was extracted with with 75% aqueous alcohol (2 x 4 l) and the combined extracts were concentrated under vacuum; the residue was purified by ion exchange chromatography.[3]



3,8-Diepialexine was eluted after castanospermine from Amberlite CG 120 (NH_4^+ form) by 0.1 M ammonium hydroxide to give 3,8-diepialexine as an oil; the alkaloid (1) crystallised readily from aqueous ethanol as the hydrochloride salt, (0.32 g), m.p. 148°-152°C, $[\alpha]^{20}$ (c, 1.35 in H_2O),: -3.5° (589), -3.2° (578), -4.1° (546), -6.6° (436), -9.2° (365). m/z (NH_3 DCI): 190 ($M+H^+$, 100%), 158 ($M-CH_2OH^+$, 24%), 140 (85%); m/z (EI): 158 ($M-CH_2OH^+$, 50%), 72 (100%). (Found: C, 42.53; H, 6.85; N, 5.89. $C_8H_{16}NO_4Cl$ requires: C, 42.58; H, 7.16; N, 6.21%)

3,8-Diepialexine migrates on paper ionophoresis with the same mobility as alexine (0.8 and 1.1 relative to arginine at pH 1.9 and 3.6, respectively),[16] and gives a grey colour with ninhydrin reagent. The retention times of the pertrimethylsilyl derivatives of 3,8-diepialexine (1) and of alexine (2) on gas chromatography on 3% OV1 at 170°C (isothermal)[17] are 0.78 and 0.73 relative to that of the trimethylsilyl derivative of castanospermine. The proton NMR spectrum of 3,8-diepialexine hydrochloride was poorly dispersed. The proton NMR spectrum of 3,8-diepialexine as the free base was assigned on the basis of a proton-proton shift-correlation (COSY) spectrum and consisted of: (500 MHz, D_2O) δ 4.24 (1H, dt, J 2, 4.5 Hz, H-7), 4.12 (1H, t, J 3.5 Hz, H-1), 3.96 (1H, dd, J 3.5, 4.5 Hz, H-2), 3.80 + 3.70 (2H, AB part of ABX, CH_2OH), 3.25 (1H, dd, J 4, 4.5 Hz, H-8), 3.16 (1H, dt, J 4.5, 6 Hz, H-3), 2.96 (1H, ddd, J 6, 9, 11.5 Hz, H-5), 2.74 (1H, m, H-5'), 1.82 (2H, m, H-6 and H-6'). The assignment of ^{13}C NMR spectrum of 3,8-diepialexine (Table) followed from a heteronuclear shift-correlation experiment; the ^{13}C NMR spectrum of alexine (2) is given for comparison. The absolute and relative stereochemistries of 3,8-diepialexine (1) were established by an X-ray crystallographic analysis of the hydrochloride (Figure).

Table ^{13}C NMR spectra (125 MHz, D_2O) of 3,8-diepialexine and of alexine

	C-2	C-1	C-8	C-7	C-3	CH_2OH	C-5	C-6
multiplicity	d	d	d	d	d	t	t	t
3,8-diepialexine (1)	78.5	74.0	74.8	69.6	63.5	56.9	44.9	34.8
alexine (2)	76.1	75.9	70.1	69.9	64.2	58.9	45.5	34.0

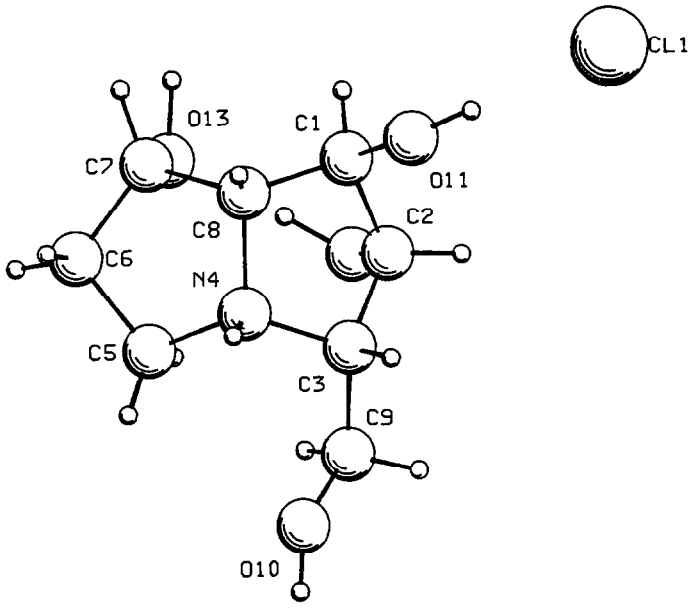


Figure. X-Ray molecular structure of hydrochloride of 3,8-diepialexine showing crystallographic numbering scheme

The potential of 3,8-diepialexine to inhibit digestive glycosidases was tested against the activity of intestinal homogenates from the mouse, guinea pig, fifth and sixth instar larvae of the Lepidopteran insect Spodoptera littoralis and the earthworm Lumbricus terrestris using both natural and synthetic subtrates (p-nitrophenyl glycopyranosides).[18] Intestinal homogenates were prepared as previously reported;[19] the homogenates were dialysed against distilled water before use. For natural substrates, the method of assay was essentially that of Dahlqvist;[20] the substrate (28 mM), inhibitor and suitably diluted intestinal homogenate were incubated for 1 h at 37°C in maleate buffer (50 mM, pH 6.0) after which the reaction was terminated by the addition a tris-glucose oxidase reagent at pH 7.0[21] providing an assay for the liberated glucose. The same assay conditions were used with the synthetic substrates, except that the substrate concentration was 5 mM and the reaction was stopped by addition of 0.4 M glycine-sodium hydroxide buffer, pH 10.7. In addition to the intestinal digestive glycosidases, hetero-β-galactosidase activity of guinea pig small intestine and the β-glucosidase activity of Penicillium expansum were assayed using synthetic substrates as above and the sucrase activity of bakers' yeast (Saccharomyces cerevisiae) was assayed using sucrose as substrate.

3,8-Diepialexine inhibited the hydrolysis of the corresponding p-nitrophenyl glycopyranosides by less than 50% at a concentration of 3.3×10^{-4}M (α-glucosidase of the mouse, S. littoralis and earthworm; β-glucosidase of the mouse, S. littoralis and P. expansum; α-galactosidase of earthworm; β-galactosidase of mouse and S. littoralis; hetero-β-galactosidase of guinea pig; α-mannosidase of guinea pig and earthworm; β-mannosidase of S. littoralis). Inhibition of natural substrate hydrolysis was less than 50% at a concentration of 3.3×10^{-4}M (maltase, lactase and trehalase of mouse; maltase, lactase, trehalase and sucrase of S. littoralis; sucrase of yeast). Inhibition of maltase activity was particularly poor. The only significant inhibition observed was that of mouse sucrase where the inhibitor (1) concentration giving 50% inhibition of hydrolysis (IC_{50}) was 2.1×10^{-4}M; alexine (2) caused about 30% inhibition of mouse sucrase activity at 3.3×10^{-4}M.[22] In contrast, inhibition of β-galactosidase activity of mouse intestine against p-nitrophenyl β-galactopyranoside by alexine was greater (IC_{50} 1.5×10^{-4}M) than by 3,8-diepialexine (about 36% inhibition at 3.3×10^{-4}M).

Like alexine (2),[2] 3,8-diepialexine (1) is a poor inhibitor of glycosidase activity when compared with monocyclic polyhydroxylated pyrrolidines such as DMDP (6).[14,19] In summary, although both of the pyrrolizidine alkaloids are weak inhibitors of glycosidase activity, 3,8-diepialexine is a somewhat better inhibitor of mammalian α-glucosidase activity than is alexine, while alexine is a better inhibitor of ß-glucosidase activity.

X-Ray Crystal Structure Analysis. The structure of the hydrochloride of 3,8-diepialexine (Figure), including the absolute configuration, was established by single crystal X-ray analysis. Cell dimensions and intensity data were measured with an Enraf-Nonius CAD4-F diffractometer up to $\theta = 75^{\circ}$ (Cu-Kα radiation). The data were corrected for absorption, Lorentz and polarisation effects. All calculations were carried out on a VAX 11/750 computer using SHELXS-86[23] for direct methods and CRYSTALS[24] for all other calculations. Atomic scattering factors were taken from International Tables.[25] The coordinates of all non-hydrogen atoms were given by SHELXS-86. A subsequent difference Fourier map revealed all the hydrogen atoms. The structure was refined by full-matrix least squares; a correction for secondary extinction[26] was also refined. The data were then re-reduced without applying Friedel's law. The Flack enantiopole parameter[27] then refined to 0.00(3), indicating that the absolute configuration is that shown in the Figure. The original data reduction was then redone and weights calculated from a modified Chebyshev weighting scheme.[28] Atomic coordinates have been deposited.[29]

Crystal data for 3,8-diepialexine hydrochloride (1):

Formula	$C_8H_{15}NO_4.HCl$
M_r	225.67
Crystal size / mm	0.33 x 0.43 x 0.63
Crystal system	orthorhombic
a/Å	7.539(1)
b/Å	11.310(1)
c/Å	12.427(2)
α/°	90
ß/°	90
γ/°	90
U/Å^3	1059.7
space group	$P2_12_12_1$
Z	4
D_c/g cm^{-3}	1.415
F(000)	480
μ/ cm^{-1}	31.90
Radiation	Cu-Kα
$(\sin\theta/\lambda)_{max}$	0.63246
Total data	1968
Total unique data	1280
Observed data [I>3σ(I)]	1242
Absorption correction:	min 1.93, max 2.41
Merging R	2.80%
No. of parameters	192
Weights	15.4, -4.9, 10.3
Extinction parameter	26(5)
Final Σ(shift/error)^2	0.04
Maximum residual electron density/ eÅ^{-3}	0.05
R	3.41%
Rw	3.96%

Fractional atomic coordinates and equivalent isotropic* temperature factors with e.s.d.s in parentheses (atomic labelling as in FIGURE)

Atom	x/a	y/b	z/c	U(iso)
Cl(1)	0.1685(1)	0.27089(7)	0.25867(5)	0.0477
C(1)	-0.0570(4)	0.0732(2)	0.4485(2)	0.0385
C(2)	0.1184(4)	0.0649(2)	0.5105(2)	0.0384
C(3)	0.0637(3)	0.0906(2)	0.6261(2)	0.0356
N(4)	-0.1171(3)	0.0340(2)	0.6371(1)	0.0298
C(5)	-0.1326(4)	-0.0858(2)	0.6897(2)	0.0402
C(6)	-0.2877(4)	-0.1404(3)	0.6294(3)	0.0508
C(7)	-0.2480(4)	-0.1073(3)	0.5139(2)	0.0445
C(8)	-0.1970(3)	0.0237(2)	0.5241(2)	0.0348
C(9)	0.1882(4)	0.0566(2)	0.7155(2)	0.0465
O(10)	0.1006(3)	0.0847(2)	0.8133(2)	0.0563
O(11)	-0.1038(3)	0.1942(2)	0.4294(2)	0.0508
O(12)	0.2010(3)	-0.0475(2)	0.4988(2)	0.0465
O(13)	-0.1004(3)	-0.1760(2)	0.4776(2)	0.0512

*$U_{eq} = (U_1 x U_2 x U_3)^{1/3}$ where the mean square displacements (A^2) are along the principle axes of the thermal ellipsoid

Final anisotropic temperature factors with e.s.d.s in parentheses (atomic labelling as in FIGURE)

Atom	U(11)	U(22)	U(33)	U(23)	U(13)	U(12)
Cl(1)	0.0649(4)	0.0573(4)	0.0385(3)	0.0004(3)	-0.0047(3)	-0.0294(3)
C(1)	0.047(1)	0.039(1)	0.033(1)	0.007(1)	0.005(1)	0.006(1)
C(1)	0.040(1)	0.038(1)	0.041(1)	0.007(1)	0.009(1)	0.003(1)
C(3)	0.035(1)	0.032(1)	0.043(1)	-0.0008(9)	-0.002(1)	-0.001(1)
N(4)	0.0317(9)	0.0299(9)	0.0290(8)	-0.0018(7)	0.0005(8)	0.0031(8)
C(5)	0.053(1)	0.038(1)	0.034(1)	0.008(1)	0.003(1)	-0.004(1)
C(6)	0.055(2)	0.050(2)	0.054(2)	0.003(1)	0.005(1)	-0.016(1)
C(7)	0.047(1)	0.046(1)	0.044(1)	-0.006(1)	0.007(1)	-0.006(1)
C(8)	0.035(1)	0.039(1)	0.031(1)	0.0016(9)	-0.0009(9)	0.003(1)
C(9)	0.038(1)	0.055(2)	0.049(1)	-0.003(1)	-0.008(1)	0.002(1)
O(10)	0.057(1)	0.088(2)	0.042(1)	-0.013(1)	-0.015(1)	0.015(1)
O(11)	0.068(1)	0.044(1)	0.058(1)	0.0221(9)	0.010(1)	0.013(1)
O(12)	0.049(1)	0.054(1)	0.047(1)	0.0021(9)	0.0122(9)	0.0195(9)
O(13)	0.071(1)	0.0448(9)	0.047(1)	-0.0144(8)	-0.002(1)	0.005(1)

Bond lengths (A) for the non-hydrogen atoms with e.s.d.s in parentheses (atomic labelling as in FIGURE)

C(1)	C(2)	1.534(4)
C(1)	C(8)	1.520(3)
C(1)	O(11)	1.433(3)
C(2)	C(3)	1.522(3)
C(2)	O(12)	1.422(3)
C(3)	N(4)	1.512(3)
C(3)	C(9)	1.505(4)
N(4)	C(5)	1.509(3)
N(4)	C(8)	1.533(3)
C(5)	C(6)	1.520(4)
C(6)	C(7)	1.514(4)
C(7)	C(8)	1.537(4)
C(7)	O(13)	1.430(4)
C(9)	O(10)	1.419(3)

Bond angles (°) for the non-hydrogen atoms with e.s.d.s in parentheses (atomic labelling as in FIGURE)

C(8)	C(1)	C(2)	105.4(2)
O(11)	C(1)	C(2)	110.8(2)
O(11)	C(1)	C(8)	106.4(2)
C(3)	C(2)	C(1)	103.2(2)
O(12)	C(2)	C(1)	112.4(2)
O(12)	C(2)	C(3)	112.7(2)
N(4)	C(3)	C(2)	104.4(2)
C(9)	C(3)	C(2)	118.6(2)
C(9)	C(3)	N(4)	112.8(2)
C(5)	N(4)	C(3)	119.3(2)
C(8)	N(4)	C(3)	107.6(2)
C(8)	N(4)	C(5)	107.4(2)
C(6)	C(5)	N(4)	102.1(2)
C(7)	C(6)	C(5)	102.5(2)
C(8)	C(7)	C(6)	102.1(2)
O(13)	C(7)	C(6)	108.6(3)
O(13)	C(7)	C(8)	110.8(2)
N(4)	C(8)	C(1)	105.4(2)
C(7)	C(8)	C(1)	118.5(2)
C(7)	C(8)	N(4)	104.3(2)
O(10)	C(9)	C(3)	106.6(2)

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