Tetrahedron Letters,Vol.29,No.20,pp 2487-2490,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Press plc

ISOLATION FROM ALEXA LEIOPETALA AND X-RAY CRYSTAL STRUCTURE OF ALEXINE, (1R,2R,3R,7S,8S)-3-HYDROXYMETHYL-1,2,7-TRIHYDROXYPYRROLIZIDINE [(2R,3R,4R,5S,6S)-2-HYDROXYMETHYL-1-AZABICYCLO[3.3.0]OCTAN-3,4,6-TRIOL], A UNIQUE PYRROLIZIDINE ALKALOID

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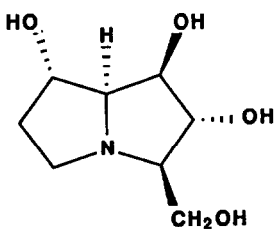
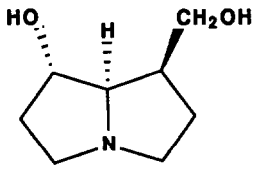
The isolation from Alexa leiopetala and identification by X-ray crystal structure analysis of (1R,2R,3R,7S,8S)-3-hydroxymethyl-1,2,7-trihydroxypyrrolizidine [(2R,3R,4R,5S,6S)-2-hydroxymethyl-1-azabicyclo[3.3.0]octan-3,4,6-triol], a unique pyrrolizidine alkaloid, is described. A preliminary study of the inhibition of glycosidases by alexine is reported.

This paper reports the isolation from Alexa leiopetala and identification of (1R,2R,3R,7S,8S)-3-hydroxymethyl-1,2,7-trihydroxypyrrolizidine (1), which has been given the trivial name alexine. Although there are many pyrrolizidine alkaloids with a carbon substituent at C-1,[1] such as dihydroxyheliotridane (2),[2] (1) is the first example of a pyrrolizidine alkaloid with a carbon substituent at C-3. There is also a significant structural resemblance to 2R,5R-dihydroxymethyl-3R,4R-dihydroxypyrrolidine, DMDP (3) an alkaloidal glucosidase inhibitor[3] which occurs in some spp. of Derris and Lonchocarpus (Leguminosae).[4] Alexa spp. (Sophoreae, Leguminosae) are trees native to the wet lands of Guyana, Surinam, French Guiana, Venezuela and the Amazon basin; no alkaloids have previously been reported to occur in this genus.



(1)



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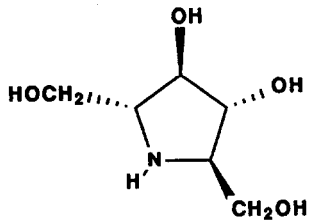

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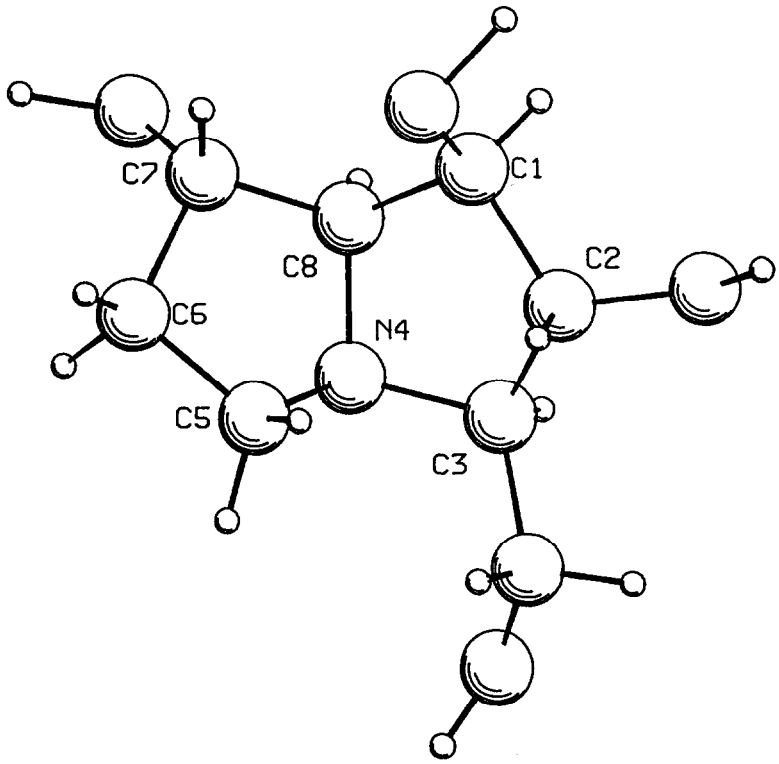

FIGURE 1

Isolation. Finely ground dried pod of Alexa leiopetala Sandwith (Davis) 1065 (11g) was extracted with 75% aqueous ethanol (3 x 55 ml) and the combined extracts were concentrated under vacuum; the residue was purified by ion exchange chromatography.[5] Alexine (1) (70 mg) was readily crystallised from aqueous ethanol as cubic-shaped crystals, m.p. 162°-163°C, $[\alpha]_D^{20}$ +40.0° (c, 0.25 in H_2O), m/z (NH_3 DCI): 190 ($M+H^+$, 100%); m/z (EI): 158 ($M-CH_2OH^+$, 100%). Alexine (1) migrates on paper ionophoresis with a mobility of 0.8 relative to arginine[5] and in gas chromatographic studies on 3% OV1 at 170°C (isothermal), the trimethylsilyl derivative of (1) was shown to have a retention time of 1.78 relative to the trimethylsilyl derivative of DMDP (3).[6] The ^1H NMR spectrum of (1), assigned on the basis of a homonuclear shift correlation (COSY) experiment, consisted of (500 MHz in D_2O): δ 4.29 (1H, m, H-7), 4.05 (1H, dd, J 6.2, 7.8 Hz, H-1), 3.69 (2H, AB part of ABX, $C\underline{H}_2OH$), 3.64 (1H, dd, J 6.2, 9.0 Hz, H-2), 3.19 (1H, dd, J 5.5, 7.8 Hz, H-8), 2.79 (3H, m, H-3, H-5, H-5'), 2.04 and 1.60 (2 x 1H, m, H-6, H-6'). The assignments of the ^{13}C NMR spectrum followed from a heteronuclear shift correlation experiment (125 MHz in D_2O): δ 76.1 (d, C-2), 75.9 (d, C-1), 70.1 (d, C-8), 69.9 (d, C-7), 64.2 (d, C-3), 58.9 (t, $C\underline{H}_2OH$), 45.5 (t, C-5) and 34.0 (t, C-6).

The structure of (1), including the absolute configuration, was established by X-ray crystallography.[7] Cell dimensions and intensities were measured with an

Enraf-Nonius CAD4 diffractometer up to θ = 66.5° (CuKα radiation). 2807 reflections [I>3σ(I)] (Friedel pairs not merged) were used in the analysis. The structure was solved by direct methods with SHELXS[8] and refined by full-matrix least squares with CRYSTALS;[9] heavier atoms anisotropic; hydrogen atoms were located from difference maps but their coordinates were not refined. The absolute configuration was determined by refinement of the Rogers[10] and Flack[11] enantiomorph-polarity parameters which converged to 0.98(27) and 0.19(14),

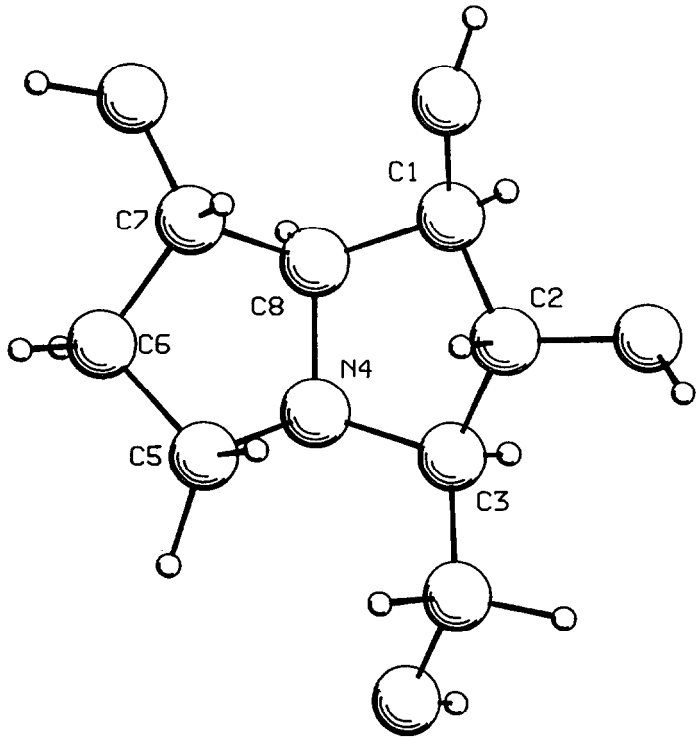

FIGURE 2

respectively, establishing the configuration shown in the Figures. The final R and Rw values are 0.037 and 0.042. Atomic coordinates have been deposited.[12]
The two independent molecules in the crystal asymmetric unit (Figures 1 and 2), although chemically identical, differ somewhat in conformation. Thus in Figure 1, the C1-N-C8 ring adopts a half-chair conformation with the nitrogen atom and C3 displaced by 0.28A on opposite sides of the C8-C1-C2 plane. The other five membered ring is in the envelope conformation with C5 positioned 0.59A from the plane of the four atoms C6-C7-C8-N. The other molecule, in Figure 2, has a more symmetrical geometry; both rings are envelopes with C2 (by 0.58A) and C6 (by 0.68A) as the out of plane atoms. The differences in conformation may be attributed to crystal packing interactions which involve a network of strong O-H...N and O-H...O hydrogen bonds. The pyrrolizidine ring system thus shows a considerable degree of flexibility, allowing quite large movements of the substituents.

The structural resemblance of alexine (1) to DMDP (3) suggested that alexine may also have glycosidase-inhibitory properties. A study of the effects of alexine on digestive glucosidases of mouse small intestine using either 6mM p-nitrophenyl-α-D-glucopyranoside or 10mM p-nitrophenyl-ß-D-glucopyranoside as substrate revealed that alexine inhibited the hydrolysis of both substrates by less than 50% at 3.3 x 10^{-4}M; alexine inhibited the hydrolysis of 10mM p-nitrophenyl-ß-D-galactopyranoside by 50% at 1.5 x 10^{-4}M. Under similar conditions, DMDP (3) was previously demonstrated to inhibit the hydrolysis of these substrates by 50% at concentrations of 3.0 x 10^{-4}M, 1.0 x 10^{-5}M, and 2.0 x 10^{-6}M respectively;[13] these studies indicate that alexine is a poor inhibitor of mammalian digestive ß-glucosidase and ß-galactosidase in comparison to DMDP (3). Alexine (1) was also shown to have no effect on ß-glucosidase of Penicillium expansum under conditions where DMDP (3) caused 50% enzymic inhibition at 1.4 x 10^{-5}M.[14] Further studies of the biological evaluation of alexine as a glycosidase inhibitor are in progress.

Acknowledgments. TAH was on leave from the Department of Chemistry, University of Birmingham; we thank the Bentham-Moxon Trust and the Medical Research Council for financial support (to RJN) and Charles Stirton for taxonomic guidance.

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14. Substrate 10mM p-nitrophenyl-ß-D-glucopyranoside, buffer 50 mM maleate, pH 6.0.