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ISOLATION OF 7A-EPIALEXAFLORINE FROM LEAVES OF *ALEXA GRANDIFLORA* A UNIQUE PYRROLIZIDINE AMINO ACID WITH A CARBOXYLIC ACID SUBSTITUENT AT C-3

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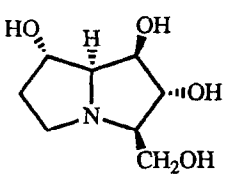
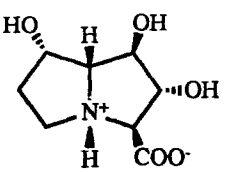
The isolation and identification by X-ray crystal structure analysis of 7a-epialexaflorine (1R,2R,3S,7S,7aR)-3-carboxy-1,2,7-trihydroxypyrrolizidine, the first example of a naturally occurring pyrrolizidine-3-carboxylic acid, from *Alexa grandiflora* is described.

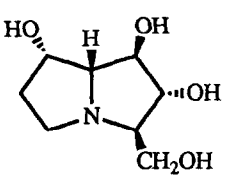
The pyrrolizidine alkaloid, alexine [(1R,2R,3R,7S,7aS)-3-hydroxymethyl-1,2,7-trihydroxypyrrolizidine] (1), isolated from *Alexa leiopetala*,[1] was the first example of a pyrrolizidine alkaloid having a carbon substituent at C-3 in comparison to the more commonly found necine bases with the carbon substituent at C-1.[2] Subsequently several other alexines have been isolated[3,4] or synthesized[5] and shown to be inhibitors of glycosidases.[6] The alexines occur not only in all species of the legume genus *Alexa* but also in the related species *Castanospermum australe*. Both genera also contain the tetrahydroxyoctahydroindolizine castanospermine,[7] a potent glucosidase inhibitor which is also inhibitory to HIV.[8] We here report the isolation and identification of 7a-epialexaflorine (2), (1R,2R,3S,7S,7aR)-3-carboxy-1,2,7-trihydroxypyrrolizidine, the first example of an amino acid with the carboxyl substitutent at C-3 of the pyrrolizidine nucleus; the stereochemistry of 7a-epialexaflorine corresponds to that of 7a-epialexine (australine).



alexine (1)



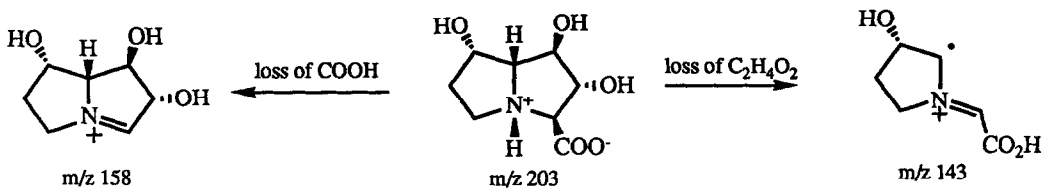
7a-epialexaflorine (2)



7a-epialexine (3)

Isolation and characterisation.

Leaves of *Alexa grandiflora* Ducke were collected from a specimen growing in the zoobotanic garden of Museu Paraense Emilio Goeldi, Pará. Dried powdered leaves (645 g) were defatted by percolation with petrol (removal of which gave residue A, 7.5 g) and then exhaustively percolated with chloroform (giving residue B, 17.6 g), and finally extracted with ethanol (affording residue C, 114.6 g). Residues A and B yielded steroids 25(27)-dehydroporiferasterol (37 mg) and clerosterol (16 mg) and the triterpenoid lupenone (15 mg) on elution from silica with hexane-ethyl acetate. Concentration of the mother liquor from the ethanol extraction gave a precipitate which was filtered and then readily crystallised from methanol-water (1:1) (1.7 g). These crystals were dissolved in water and, after cation exchange chromatography with elution by 2M aqueous ammonium hydroxide, gave pure 7a-epialexaflorine (2), $[\alpha]^{20}$ (*c*, 0.3 in H_2O); -0.4 (589), -0.7 (578), -1.5 (546), -1.1 (436), -1.1 (365).[9]



Scheme. Mass spectral (EI) fragmentation of alexaflorine (2)

7a-Epialexaflorine does not melt up to 270°C (dec. 230°C), ν_{max} (KBr): 3310-2880, 1660 (C=O), 1400 cm^{-1}; m/z (EI): 203 (M^+, 1%); 158 (M-CO_2H^+, 100%); 143 (M-$C_2O_2H_4^+$, 67%); 126 (33%); m/z (DCI NH_3); 204 ($M+H^+$, 100%), 186 ($M+H-H_2O^+$, 24%), 170 (23%), 158 (M-CO_2H^+, 32%), 150 (65%), 124 (80%), 106 (72%); δ_H (D_2O): 2.10 (2H, m, H-6, H-6'), 3.20 (1H, m, H-5), 3.65 (1H, m, H-5'), 3.72 (1H, d, H-3, $J_{2,3}$ 7.2 Hz), 3.93 (1H, dd, H-7a, $J_{7,7a}$ 5.2 Hz, $J_{1,7a}$ 5.2 Hz), 4.23 (1H, dd, H-2, $J_{1,2}$ 5.2 Hz), 4.34 (1H, dd, H-1), 4.53 (1H, m, H-7); δ_C (125 MHz in D_2O with MeOH as internal standard): 172.0 (s, COOH), 80.7 (d), 75.2 (d), 74.3 (d), 74.2 (d), 69.7 (d), 54.3 (t, C-5), 35.1 (t, C-6) (Found: C, 46.96; H, 6.35; N, 6.77; $C_8H_{13}NO_5$ requires: C, 47.29; H, 6.45; N, 6.89%).

7a-Epialexaflorine (2) migrates on paper ionophoresis with a mobility of 0.3 relative to arginine at pH 1.9 and gives a grey colour with ninhydrin reagent. The insolubility of (2) in all solvents except water, its resistance to melting and the infrared evidence for a carboxylate ion provide evidence for the existence of (2) in zwitterionic form. The mass spectral data (EI) may be explained by the fragmentation shown (Scheme). All the data for 7a-epialexaflorine, including the ^1H and ^{13}C NMR spectra are consistent with the proposed structure; however, it is not possible to determine with confidence the relative configuration of (2) from these NMR experiments and so both the absolute and relative stereochemistries of 7a-epialexaflorine were established by single crystal X-ray crystallographic analysis (Figure).[10]

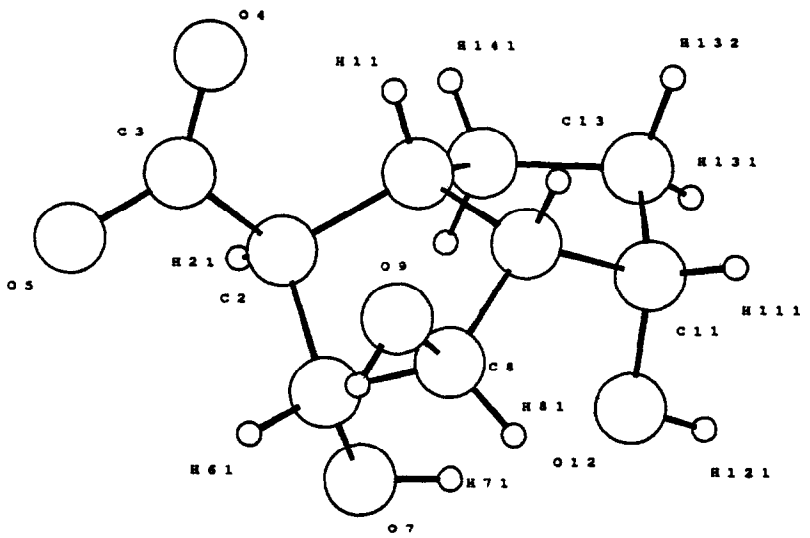


Figure. X-Ray molecular structure of 7a-epialexaflorine (2), showing crystallographic numbering scheme.

Enzyme inhibition studies

7a-Epialexaflorine (2) was found to cause 50% inhibition of fungal amylglucosidase (glucan 1,4-α-glucosidase from *Aspergillus niger*) in 50 mM maleate buffer (pH 6) at a concentration of 1.4 x 10^{-4}M. This inhibition was much weaker than that shown by many other hydroxylated alkaloids.[6] There was less than 50% inhibition at a concentration of 3.3 x 10^{-4}M against a wide range of other enzymes (including α-glucosidase from intestines of the mouse and larvae of the insects *Spodoptera littoralis* and *Heliconius melpomone*; β-glucosidase from the mouse, *H. melpomone* and *Penicillium expansum*; α-mannosidase from Jack bean, the aphid *Myzus persicae* and guinea pig intestine and α-amylase from pig pancreas and *Aspergillus oryzae*). 7a-Epialexaflorine was slightly active against mouse intestinal sucrase (48% inhibition at 3.3 x 10^{-4}M).

X-Ray Crystal Structure Analysis.

The structure of 7a-epialexaflorine (1R,2R,3S,7S,7aR)-3-carboxy-1,2,7-trihydroxypyrrolizidine (2) (crystallised from methanol-water), molecular formula $C_8H_{13}NO_5$; formula weight 203.194 was established by single crystal X-ray analysis. Cell dimensions and intensity data were measured with an Enraf-Nonius CAD4-F diffractometer up to θ = 75° (Cu-Kα radiation). The data were corrected for absorption, Lorentz and polarisation effects. All calculations were carried out on a Microvax 3800 computer using SHLEXS-86[11] for direct methods and CRYSTALS[12] for all other calculations. Atomic scattering factors were taken from International Tables.[13] Atomic coordinates for the amino acid (2) have been deposited at the Cambridge Crystallographic Data Centre.[10] The coordinates of all non-hydrogen atoms were given by SHELXS-86. The hydrogen atoms were placed geometrically except for the hydroxyl and amino hydrogens which were found by Fourier difference maps. The structure was refined by full-matrix least-squares with isotropic temperature factors for the hydrogen atoms and anisotropic temperature factors for all other atoms using data with unmerged Friedel pairs. Corrections for secondary extinction were applied,[14] and the models refined almost to convergence. The data were refined using Chebyshev weighting schemes[15] to give a final value of R = 3.49%. The absolute configuration was determined from the Flack Enantiopole which was refined to a value of 0.0(1).

Crystal data:	Crystal system: Monoclinic
a/Å 6.9814	α/° 90
b/Å 7.8609	β/° 114.96
c/Å 15.5814	γ/° 90
Space group $P2_1$	D_C/g cm^{-3} 1.5786
linear absorption coeff. /cm^{-1} 10.8206	Crystal size /mm 0.3 x 0.7 x 1.0
Data collection:	
X-radiation λ = 1.5418 Å Cu-Kα	θ min., max. /° 0 - 75
ω-scan parameters: A, B (°) (A + B tanθ) A = 1.30 B = 0.15	
Horizontal aperture parameters: A, B (mm) (A + B tanθ) A = 3.0 B = 0.0	
Scan speed/° min^{-1} 2.2 (min) 6.7(max)	Total data 4880
Observed data 1693 for [I>nσ(I)] where n = 3	
Absorption correction: 1.33 (min) 1.66 (max)	Merging R 3.53 %
Refinement: Solved by SHELXS-86	
Weighting Scheme type Chebyshev 14 3	Weights 14.2, -1.77 , 10.5
Extinction parameter 91(4)	
Maximum residual electron density/ eÅ^{-3} 0.39	Flack Enantiopole 0.0(1)
Final R 3.18 %	R_w 3.50 %

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9 Wavelengths at which the specific rotations were determined are given in parenthesis.
10 The atomic coordinates for 7a-epialexaflorine (2) are available on request from the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW; the crystallographic numbering system differs from that used elsewhere in the text. Any request should be accompanied by the full literature citation for this paper.
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