Isolation of the pyrrolizidine alkaloid 1-epialexine from *Castanospermum australe*

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\textbf{A B S T R A C T}

The Australian leguminous tree *Castanospermum australe* contains the anti-viral glucose analogue indolizidine alkaloid castanospermine. As the result of a search for new bioactive carbohydrate-like compounds we now report the isolation of the novel polyhydroxylated pyrrolizidine alkaloid, 1-epialexine, from the leaves and stems of *C. australe*. 1-Epialexine is a weak inhibitor of \(\beta\)-mannosidase from *Cellulomonas fimi*.

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\textbf{1. Introduction}

*Castanospermum australe* is a rich source of tetrahydroxylated alkaloids such as castanospermine, which has attracted considerable interest as a glucosidase inhibitor and potential anti-viral agent (Watson et al., 2001). Alexine, isolated from the very closely related leguminous tree *Alexa leiopetala* (Nash et al., 1988a), was the first example of a class of rare 3-hydroxymethyltrihydroxypyrrolizidines. C-7a Epimeric alexines (australines) have been reported from *C. australis* (Watson et al., 2001) and the 3-hydroxymethylhydroxypyrrolizidines are attracting interest as immune modulators (Watson et al., 2001). Inosugar analogues such as the alexines and australines have considerable potential as therapeutic agents (Watson et al., 2001) and the 3-hydroxymethylhydroxypyrrolizidines are attracting interest as immune modulators (Watson et al., 2001).

The positive response to chlorine-o-tolidine reagent suggested that the compound isolated was an alkaloid. The \(^{13}\text{C}\) NMR spectroscopic data revealed the presence of three methylene (\(\delta 33.5, 44.6\) and 59.1) and five methine (\(\delta 64.4, 71.0, 73.8, 74.3\) and 75.6) carbon atoms. The connectivity of the carbon and hydrogen atoms was defined from COSY and HMBC spectroscopic data. Two methine (\(\delta 64.4\) and 75.6) and one methylene (\(\delta 44.6\)) signals with relatively down-field chemical shifts were suggestive of being bonded to the nitrogen of the pyrrolizidine ring. The methylene signal at \(\delta 59.1\) (C-8) was attributed to the hydroxymethyl carbon; this showed HMBC correlations to \(\delta 71.0\) (C-2) and 64.4 (C-3). The methine signal at \(\delta 64.4\) (C-3) showed a HMBC correlation to \(\delta 44.6\) (C-5) confirming the pyrrolizidine structure. The absolute configurations of which were confirmed by X-ray crystallography (Thompson et al., 2008).

\textbf{2. Results and discussion}

The analysis of the structures of alexines is not simple (Wormald et al., 1998; Kato et al., 2003), and confirmation of structure by X-ray crystallographic analysis is essential to ensure that structures are properly reported. We now report on the isolation and identification of 1-epialexine, the relative and
H-7 indicated that H-1, H-2 and H-7 were on the same side of the ring. There was also a correlation to the overlapping signals of H-7a and H-3.

From the data two structures are possible either 1-epialexine (Fig. 1) or 1,7a diepialexine. The absolute and relative configurations of 1-epialexine (1) were determined by X-ray crystallographic analysis, establishing the structure as (1S,2R,3R,7S,7aS)-1,2,7-trihydroxy-3-(hydroxymethyl)pyrrolizidine (1-epialexine) (Thompson et al., 2008). Alexine is a moderate glucosidase inhibitor but 1-epialexine showed no strong inhibition of several glucosidases with the most potent inhibition being against β-mannosidase (40% at 0.8 mM).

This is the first alexine alkaloid to be reported from C. australe that has the same orientation at C-7a as alexine. Alexine itself was not detected in the C. australe extract. Although both related legume genera produce the tetrahydroxyindolizidine alkaloid castanospermine as a major alkaloid (Nash et al., 1988b), the C. australe that has the same orientation at C-7a as alexine. Alexine itself was not detected in the C. australe extract. Although both related legume genera produce the tetrahydroxyindolizidine alkaloid castanospermine as a major alkaloid (Nash et al., 1988b), the monotypic C. australe native to Australia produces predominantly australines and the South American Alexa species produce alexines. Both genera produce a cocktail of tetrahydroxylated indolizidine and pyrrolizidine alkaloids that have not as yet been found to be produced by any other organisms. The alkaloids inhibit a range of glycosidases but except for castanospermine few of the alkaloids from these plants have been widely studied for therapeutic activity. Castanospermine has a wide range of potential therapeutic activities due to glucosidase inhibition but it is quite nonspecific in which glucosidases it inhibits leading to side effects such as gastrointestinal disturbance (Watson et al., 2001). It is probably an advantage to the plants to have a mixture of compounds inhibiting a wide range of glycosidases that will reduce nutritional value to herbivores and the mixture will also reduce the chances of quick development of resistance. The individual compounds may well have therapeutic advantages over castanospermine by being more selective in inhibitory profile to glycosidases. Such compounds may also interact with carbohydrate receptors without glycosidase inhibition. Further isolation work on both genera could be profitable as both Alexa and Castanospernum contain other related, as yet unreported, compounds detected by GC–MS. No research has been reported on such alkaloids in some of the approximately 9 Alexa species in South America. The apparent presence of these alkaloids only in Alexa species in tropical South America and C. australe in tropical Australia suggests a common ancestor occurring in the tropical regions of Gondwana prior to division of the current continents.

3. Experimental

3.1. General experimental procedures

The purity of samples was checked by HPTLC on silica gel 60F254 (E. Merck) using the solvent system PrOH–AcOH–H2O (4:1:1), and a chlorine-o-tolidine reagent or iodine vapour was used for detection. 1H NMR (500 MHz) spectra and 13C NMR (125 MHz) were recorded on a Bruker DRX500 spectrometer. Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)propionate (TSP) in D2O as internal standard. The assignment of proton and carbon NMR signals was determined from extensive homonuclear decoupling experiments and the DEPT, 1H–1H COSY, HMQC and HMBC spectroscopic data. FABMS were recorded using glycerol as a matrix on a JEOL JMS-700 spectrometer. For GC–MS analyses, samples were freeze dried and prepared as pertrimethylsilyl ethers at 60 °C for 20 min using Tri-Sil reagent (Pierce Biotechnology Inc., Rockford, IL). The column was a 25 m × 0.25 mm VF-5ms “Factor Four” (film thickness, 0.25 μm) capillary column (Varian Inc.), and the 25 min temperature program ran from 160 to 300 °C with an initial rate of increase of 10 °C/min and then held at 300 °C. The mass spectrometer was a Perkin-Elmer TurboMass Gold, with a quadrupole ion filter system, which was run at 250 °C constantly during analysis, and the mass range was set to 100–650 amu.

Glycosidase assays were carried out using 0.8 mM 1-epialexine on the following enzymes: α-α-glucosidase (Saccharomyces cerevisiae and Bacillus stearothermophilus), almond β-α-glucosidase, α-α-galactosidase (Green coffee beans), bovine liver β-α-galactosidase, jack bean α-α-mannosidase, β-β-mannosidase (Cellulomonas fimi), N-acetyl-β-α-galactosaminidase (bovine kidney and Jack bean) and β-glucuronidase (bovine liver). All enzymes and para-nitrophenol substrates were purchased from Sigma, with the exception of beta-mannosidase which came from Megazyme. Assays were carried out in triplicate as described in Watson et al. (1997).

3.2. Extraction and isolation

3 kg of the leaves and stems of C. australe were homogenized in 50% aqueous EtOH. The filtrate was applied to a column of Amberlite IR-120B (2000 ml, H+ form), washed with 50% aqueous EtOH followed by water and eluted with 2N NH4OH. The ammonium hydroxide eluate was concentrated to give a brown oil, which was applied to Amberlite CG-50 (NH4+ form) to remove amino acids and pigments, and eluted with H2O. This eluate was concentrated and chromatographed repeatedly over an Amberlite CG-400 column with water as eluant to give 1-epialexine (1). The fractionation was monitored by GC–MS analysis with alexines giving characteristic tms mass spectra with fragment ions at m/z 258, 374 (base peak) and 462. The retention time was 11.0 min with the major indolizidine alkaloid of the plant, castanospermine, running at 11.3 min and 6-epicastanospermine at 10.7 min. The castanospermines gave characteristic ions as tms derivatives at m/z 361 and 387. 150 mg of 1-epialexine was purified.

3.2.1. 1-Epialexine (1)

1H NMR (500 MHz, D2O) δ/ppm 1.70 (1H, m, H-6a), 2.09 (1H, m, H-6b), 2.83 (1H, m, H-5a), 2.93 (1H, m, H-5b), 3.10 (1H, dd, J = 2.5, 6.0 Hz, H-7a), 3.13 (1H, ddd, J = 5.04, 7.3, 9.5 Hz, H-3), 3.81 (2H, m, H-8), 3.88 (1H, dd, J = 5.0, 9.5 Hz, H-2), 4.00 (1H, dd, J = 2.5, 5.4 Hz, H-1); 13C (126 MHz, D2O) δ/ppm 33.5 (C-6), 44.6 (C-5), 59.1 (C-8), 64.4 (C-3), 71.0 (C-2), 73.8 (C-1), 74.3 (C-7a); mp 159–161 °C; [α]D25 +53.4 (c, 0.43 in H2O).

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References


