Pergamon

Tetrahedron Letters, Vol. 35, No. 42, pp. 7849-7852, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01649-6

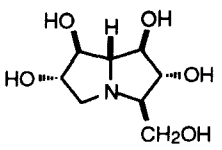
Casuarine: A Very Highly Oxygenated Pyrrolizidine Alkaloid

Robert J Nash,[a]* Paul I. Thomas,[a] Roger D. Waigh,[b] George W. J. Fleet,[c]
Mark R. Wormald,[d] Paul M. de Q. Lilley[e] and David J. Watkin[e]

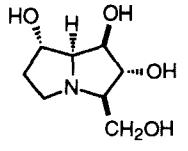
[a]Environmental Biology Department, Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB, UK; [b]Department of Pharmaceutical Sciences, University of Strathclyde, 204, George Street, Glasgow G1 1XW; [c]Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY UK; [d]Glycobiology Institute, Biochemistry Department, South Parks Road, Oxford OX1 3QU; [e]Chemical Crystallography Laboratory, Oxford University, 9 Parks Road, Oxford OX1 3PD

Abstract: The isolation from *Casuarina equisetifolia* L. (Casuarinaceae) bark of casuarine **1**, (1R,2R,3R,6S,7S,7aR)-3-(hydroxymethyl)-1,2,6,7-tetrahydroxypyrrolizidine is reported.

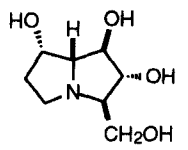
Both natural[1] and synthetic mono- and bi-cyclic nitrogen analogues of carbohydrates have potential as chemotherapeutic agents,[2] both as free bases[3] and as alkaloidal glycosides.[4,5] Alexine **2**[6] and australine **3**[7] were the first pyrrolizidine alkaloids to be isolated with a carbon substituent at C-3, rather than the more usual C-1 substituents.[8] Further stereoisomers of alexine and the related amino acid 7a-epialexaflorine **4**, found in *Alexa grandiflora*.,[9] have also been isolated The alexines and castanospermine **5** occur in all species of the genus *Alexa* and also in the related species *Castanospermum australe*; **5** is a potent glucosidase inhibitor[10] some of its derivatives may have potential for the treatment of patients with HIV.[11] Because of the reported antiviral properties of some of the alexines[12,13] and other potential applications,[14] there has been considerable interest in the synthesis of the natural products and of synthetic analogues.[15]



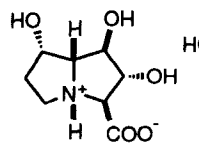
1



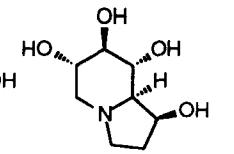
2



3



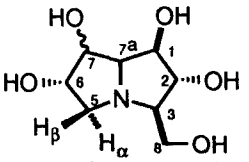
4



5

As part of a programme for the extraction of bioactive compounds from plants, this paper reports the isolation and characterisation of casuarine **1**, a more highly oxygenated analogue of **2** and **3** and at the highest oxidation level of any aminosugar analogue yet found as a natural product from any source.

Casuarina equisetifolia L. (Casuarinaceae) wood, bark and leaves have been claimed to be useful against diarrhoea, dysentery and colic.[16] A sample of bark has recently been prescribed in Western Samoa for the treatment of breast cancer; analysis by GC-MS of the pertrimethylsilylated extract of the bark revealed the presence of a pentahydroxylated pyrrolizidine alkaloid and a glycoside thereof as the major nitrogen-containing compounds present. The alkaloids were readily isolated from 75% aqueous ethanol extracts of the bark by ion-exchange chromatography using Amberlite CG120 (NH_4^+ form) by elution with 0.1 M ammonium hydroxide to give first a glycoside of casuarine and subsequently the free casuarine **1**, m.p. 181-182°C (from 95% aqueous alcohol), $[\alpha]_D^{24}$ +16.9 (c 0.8 in H_2O),[17] in 0.013% yield; the casuarine glycoside[18] was present at approximately the same concentration as the free base.



Casuarine 1 showing numbering for NMR figure and table

The ^1H NMR spectrum of casuarine **1** [Figure 1] identified[19] ten inequivalent, non-exchanging protons and eight carbons. The proton chemical shifts, multiplicities and three-bond coupling constants ($^3J_{HH}$) [from the 1D and 2D ^1H-^1H COSY spectra] and carbon chemical shifts, multiplicities and one-bond coupling constants ($^1J_{CH}$) [from the 1D and 2D ^1H-^{13}C HMQC spectra] are given in the table.

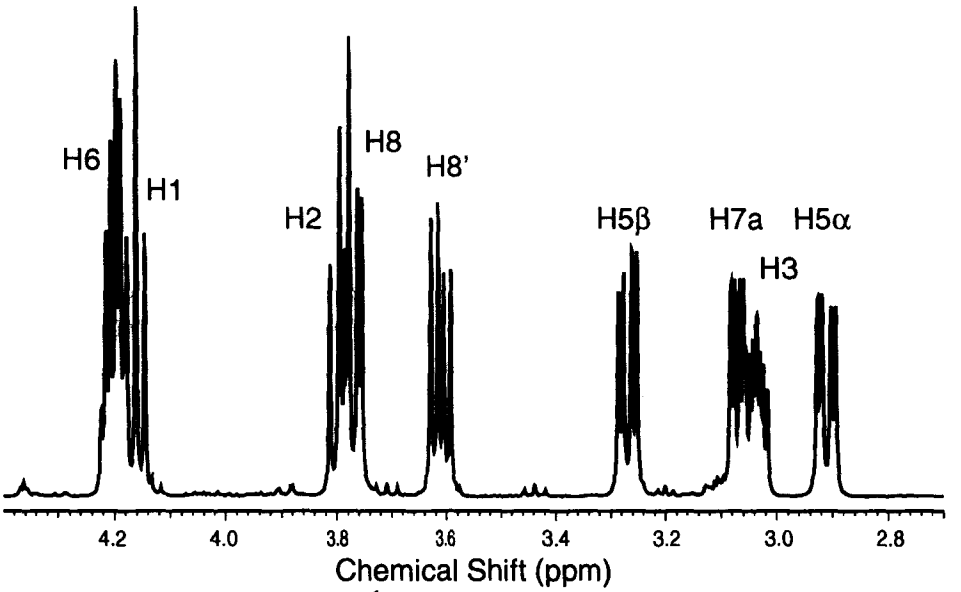


Figure 1. 500 MHz ^1H NMR spectrum of casuarine **1** in D_2O

The 2D COSY and RELAY spectra show a linear sequence of $^3J_{HH}$ correlations as follows: H5α/β -- H6 -- H7 -- H7a -- H1 -- H2 -- H3 -- H8/8' which define the carbon backbone structure. This sequence is confirmed by the $^2J_{CH}$ and $^3J_{CH}$ correlations observed in the HMBC spectrum. In addition, extra $^3J_{CH}$ correlations are observed in the HMBC spectrum as follows: C3 to H5α, H5β and H7a, C5 to H7a and possibly H3, and C7a to H5α and H5β. These define the ring structure as shown above and this is again confirmed by a ^1H-^1H NOE between H3 and H5α. Information about the relative configurations of the 6 chiral carbons may be obtained from the values of the $^3J_{HH}$ coupling constants (related to torsion angles) and ^1H-^1H NOEs (related to distances), if the ring conformations (puckering) can also be determined. The coupling constants of 8.0 Hz for H3/H2, H2/H1 and H1/H7a are only consistent with these four protons being approximately *anti* periplanar, thus defining both the relative configurations at, and ring conformation for, the ring containing these four carbons. Further confirmation of this geometry was obtained by the observation of a stronger NOE between H1 and H3 than between H1 and H2, indicating that H1 and H3 are on the same side of the ring, whilst H2 is on the opposite side. The observation of a strong NOE (corresponding to a distance of 2.5 Å or less) between H3 and H5α and no NOE between H3 and H5β indicates that H3 and H5α must be on the same side of the molecule.

Label	^1H			Label	^{13}C		
	δ (ppm)	mult	$^3J_{HH}$ (Hz)		δ (ppm)	mult	$^1J_{CH}$ (Hz)
H1	4.162	t	8.0	C1	77.77	d	146
H2	3.796	t	8.0	C2	76.63	d	139
H3	3.036	m	8.0, 3.8, 6.6	C3	69.97	d	139
H5α	2.911	dd	12.2, 4.0	C5	57.96	t	139
H5β	3.270	dd	12.2, 4.7				
H6	4.21	m	4.0, 4.7, x	C6	77.40	d	151
H7	4.19	m	x, 3.5	C7	78.79	d	148
H7a	3.071	dd	3.5, 8.0	C7a	72.09	d	146
H8	3.771	dd	11.9, 3.8	C8	62.24	t	142
H8'	3.611	dd	11.9, 6.6				

Table ^1H and ^{13}C assignments and coupling constants for casuarine in D_2O, pH=8.25 and 30°C.

A stronger NOE is observed between H6 and H5β than between H6 and H5α, indicating that H6 and H5α are on the same side of the ring. This tentatively assigns the configuration at C6 relative to C3. The coupling constant of 3.5 Hz for H7a/H7 is consistent with either a *cis* or *trans* relationship, depending on the conformation of the second ring. The H7/H6 coupling constant cannot be determined because of considerable overlap in the spectra. Thus NMR studies gave firm indications of the relative configurations at 5 of the chiral centres of casuarine **1** but could not unambiguously assign the relative configuration of the sixth stereogenic centre; an X-ray crystallographic study[20] resolved the ambiguity of the remaining centre and determined the absolute configuration of the new alkaloid (Figure 2).

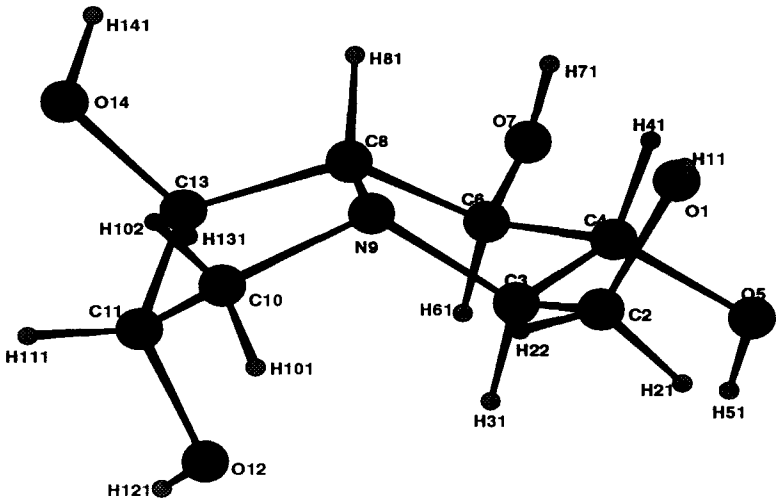


Figure 2. X-ray Molecular Structure of casuarine **1**, showing crystallographic numbering scheme

In summary this paper reports the isolation of casuarine, the first example of a pentahydroxylated pyrrolizidine alkaloid with 6 adjacent stereogenic centres and functional groups on all of the 8 carbon atoms, and a structure that provides a considerable challenge for its synthesis.[21]

REFERENCES

[1]Fellows, L. E., Kite, G. C., Nash, R. J., Simmonds, M. S. J., in Poulson, J. E., Romeo, J. J., Conn, E. E. (Eds.) *Nitrogen Metabolism of Plants*, Clarendon Press, Oxford, p.271, **1992**.

[2]Winchester, B., Fleet, G. W. J., *Glycobiology*, 1992, **2**, 199.

[3]Fischl, M. A., Resnick, L., Coombs, R., Kremer, A. B., Pottage, J. C., Fass, R. J., Fife, K. H., Powderly, W. G., Collier, A. C., Aspinall, R. L., Smith, S. I., Kowalski, K. G., Wallemark, C.-B., *J. Acquired Immune Deficiency Syndrome*, 1994, **7**, 139.

[4]Hiraizum, S., Spohr, U., Spiro, R. G., *J. Biol. Chem.*, 1993, **268**, 9927; Ardron, H., Butters, T. D., Platt, F. M., Wormald, M. R., Dwek, R. A., Fleet, G. W. J., Jacob, G. S., *Tetrahedron: Asymm.*, 1993, **4**, 2011.

[5]Asano, N., Oseki, K., Kaneko, E., Matsui, K., *Carbohydr. Res.*, 1994, **258**, 255.

[6]Nash, R. J., Fellows, L. E., Dring, J. V., Fleet, G. W. J., Derome, A. E., Hamor, T., Hegarty, M. P., Scofield, A. M., and Watkin, D. J., *Tetrahedron. Lett.*, 1988, **29**, 2487.

[7]R. J. Molyneux, M. Benson, R. Y. Wong, J. E. Tropea, and A. D. Elbein, *J. Nat. Prod.*, 1988, **51**, 1198; C. M. Harris, T. M. Harris, R. J. Molyneux, J. E. Tropea and A. D. Elbein, *Tetrahedron Lett.*, 1989, **30**, 5685.

[8]D. J. Robins, *Nat. Prod. Rep.*, 1990, **7**, 377.

[9]Pereira, A. C. de S., Kaplan, M. A. C., Maia, J. G. S., Gottlieb, O. R., Nash, R. J., Fleet, G., Pearce, L., Watkin, D. J., Scofield, A. M., *Tetrahedron Lett.*, 1991, **47**, 5637.

[10]Nash, R. J., Fellows, L. E.,. Girdhar, A., Fleet, G. W. J., Peach, J. M., Watkin, D. J., Hegarty, M. P., *Phytochemistry*, 1990, **29**, 1356.

[11]Sunkara, P. S., Taylor, D. L.,. Kang, M. S., Bowlin, T. L., Liu, P. S., Tyms, A. S.,. Sjoerdsma, A., *Lancet*, **1989**, 1206; Rhinehart, B. L., Robinson, K. M., King, C. R., Liu, P. S., *Biochem. Pharmacol.*, 1990, **39**, 1537.

[12]Fellows, L., Nash, R., *Chem. Abs.*, 1990, **114**, 143777s; Elbein, A. D., Tropea, J. E., Molyneux, R. J., *Chem. Abs.*, 1989, **113**, 91444p.

[13]Taylor, D. L., Nash, R., Fellows, L. E., Kang, M. S., Tyms, A. S., *Antiviral Chem. Chemother.*, 1992, **3**, 273.

[14]Molyneux, R. J., Pan, Y. T., Tropea, Joseph E., Benson, M., Kaushal, G. P., Elbein, Alan D., *Biochemistry*, 1990, **30**, 9981; Simmonds, M. S. J., Blaney, W. M., Fellows, L. E., *J. Chem. Ecol.*, 1990, **16**, 3167.

[15]Fleet, G. W. J., Haraldsson, M., Nash, R. J., Fellows, L. E., *Tetrahedron Lett.*, 1988, **29**, 5441; Pearson, W. H., Hines, J. V., *Tetrahedron Lett.*, 1991, **32**, 5513; Frankowski, A., Seliga, C., Bur, D., Streith, J., *Helv. Chim. Acta*, 1991, **74**, 934; Choi, S., Bruce, I., Fairbanks, A. J., Fleet, G. W., Jones, A. H., Nash, R. J., Fellows, L. E., *Tetrahedron Lett.*, 1991, **32**, 5517; Ikota, N., *Tetrahedron Lett.*, 1992, **33**, 2553.

[16]Chopra, R.N., Nayar, S. L., Chopra, I. C., *Glossary of Indian Medicinal Plants*, Council of Scientific and Industrial Research (India), New Delhi, **1956**, p. 55.

[17] For casuarine **1** - found: C, 46.59; H, 7.75; N, 6.62%. $C_8H_{15}NO_5$ requires C, 46.82; H, 7.37; N, 6.83%.

[18]The structure of the casuarine glycoside is currently under investigation.

[19]NMR abbreviations: COSY - correlation spectroscopy; HMBC - heteronuclear multiple bond correlation spectroscopy; HMQC - heteronuclear multiple quantum correlation spectroscopy; NMR - nuclear magnetic resonance; NOE - nuclear Overhauser effect; NOESY - nuclear Overhauser effect spectroscopy; RELAY - relayed correlation spectroscopy. All NMR spectra were recorded on a Varian Unity 500 spectrometer, with a probe temperature of 30°C at pH 8.25; ^1H and ^{13}C chemical shifts are referenced to trimethylsilylpropanesulphonic acid at δ 0.000and acetone at δ 29.80, respectively. All two-dimensional spectra (^1H-^1H COSY, RELAY and NOESY, ^1H-^{13}C HMQC and HMBC) were acquired in phase-sensitive mode. An 8 Hz relay step was used for the RELAY spectrum and NOESY spectra were recorded with mixing times of 100 msec to 500 msec.

[20]The atomic coordinates for casuarine 1 are available on request from the Cambridge Crystallographic Data Centre, University Chemistry Laboratory, Lensfield Road, Cambridge CB2 1EW; the crystallographic numbering system differs from that used for 1 elsewhere in the text. Any requests should be accompanied by the full literature citation for this paper.

[21]We thank Miss Marianne Sims and Mr Richard Layton for excellent technical assistance (IGER).

(*Received in UK* 18 *July* 1994; *revised* 27 *July* 1994; *accepted* 26 *August* 1994)