(1*R*,2*S*,4*S*,7a*R*)-1,2-Dihydroxy-7-thia-3a-thioniaperhydropentalene Chloride: a New, Biologically Active Pyrrolizidine Alkaloid Analogue

Aloysius H. Siriwardena,[a] Angèle Chiaroni,[a] Claude Riche,[a] Samer El-Daher,[b] Bryan Winchester[b] and David S. Grierson*[a]

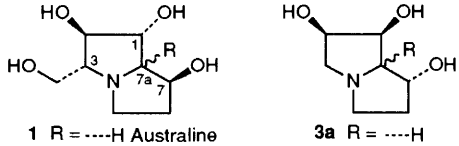
[a] *Institut de Chimie des Substances Naturelles, CNRS, 91198 Gif-sur-Yvette, France*
[b] *Division of Biochemistry and Metabolism, Institute of Child Health (University of London), 30 Guilford Street, London WC1N 1EH, UK*

The *cis*-fused bicyclic sulfonium salt **8** has been synthesized in two steps from D-erythrose and shown selectively to inhibit human liver α-mannosidases.

Isolation of the pyrrolizidine alkaloids australine **1** and alexine **2** from *Castanospermum australe* and *Alexa* species in the late 1980s has further increased the inventory of polyhydroxylated alkaloids that show glycosidase inhibition.[1–3] Synthesis of the simplified analogues **3a**,**b** lacking the C_3 hydroxymethyl side chain has subsequently been achieved in several laboratories.[4] In terms of their structure these molecules can be regarded as ring-contracted analogues of swainsonine **4** and/or bicyclic forms of the naturally occurring monocyclic pyrrolidine **5** (DIM). However, in contrast to swainsonine and DIM, which are specific and potent α-mannosidase inhibitors,[5,6] the pyrrolizidine alkaloids and their synthetic analogues are weak inhibitors of mamalian α- or β-mannosidases and β-glucosidases.[7,8]

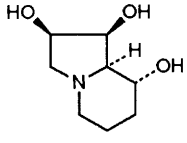
To explore further structure–activity relationships in the alexine–australine series in a search for new or enhanced biological activities we have synthesized the novel pyrrolizidine analogue **8**. Our objective in this work was to determine the combined effects on the glycosidase inhibition properties of compounds **1** to **3** of replacing the C_7 CHOH centre by a sulfur atom, and the bridgehead nitrogen by a positively charged sulfur atom. The latter modification, is a particularly important design feature, motivated by the fact that α-mannosidase inhibition by swainsonine is believed to be related to its resemblance, in its protonated form, to the mannosyl cation **9**, postulated as a high energy intermediate formed during enzyme regulated glycoside bond cleavage.[9]

The synthesis of sulfonium salt **8** involves only two steps: the preparation of the dithiolane **6** of D-(−)-erythrose and its cyclization to **8** through selective activation (tosylation) of the primary hydroxy group (Scheme 1). Although this strategy is straightforward in conception, its practical feasability was called into question by the striking scarcity of polyhydroxylated cyclic sulfonium salts reported in the literature.[10] Unsubstituted cyclic sulfonium salts have been prepared in analogous ring closure reactions,[11] but in the case of sugar dithioacetals tosylation is often difficult to control, leading to spontaneous formation of sulfonium salts only as intermediates that subsequently undergo a variety of secondary reactions. These include neutral sulfur-induced ring opening and rearrangement to thioglycosides,[12] and S_N2 reaction with their counter ion (or an added nucleophile) that results in C–SR_2^+ bond cleavage.[13,14] It is noteworthy therefore that, in spite of such inherent problems the reaction of dithiolane **6** ($[\alpha]_D$ = −15.1; *c* = 0.97, H_2O) with tosyl

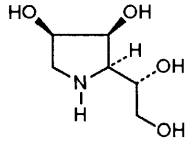


1 R = ····H Australine
2 R = ▬H Alexine

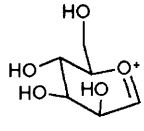
3a R = ····H
3b R = ▬H



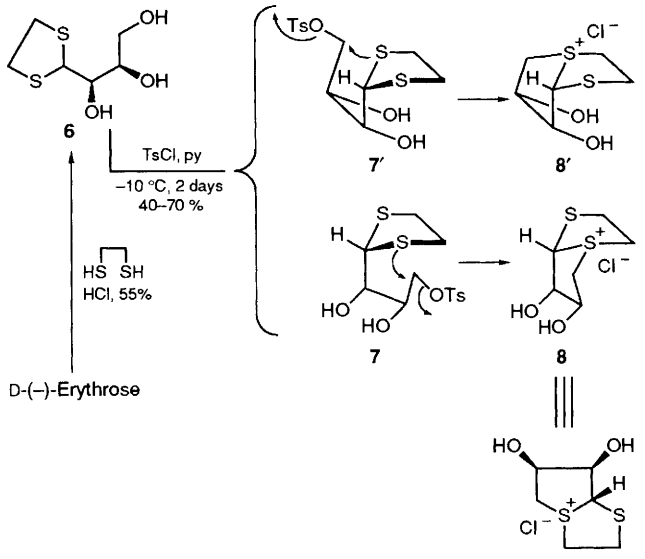
4 Swainsonine



5 DIM
(1,4-dideoxy-1,4-imino-D-mannitol)



9



Scheme 1 Ts = tosyl, py = pyridine

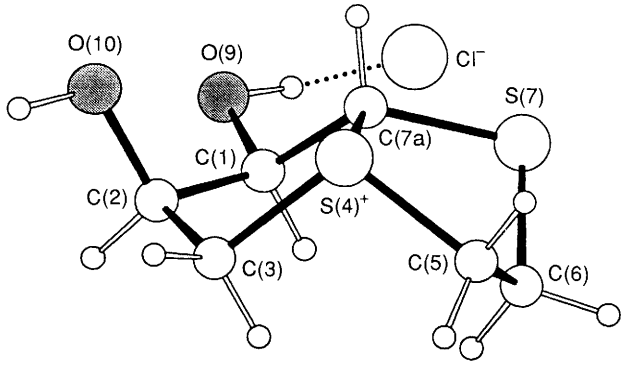


Fig. 1 ORTEP drawing of sulfonium salt **8**. Pertinent bond lengths (Å) and angles (°) include: S(4)–C(3) 1.817(3), S(4)–C(5) 1.819(4), S(4)–C(7a) 1.845(3), S(7)–C(6) 1.799(4), S(7)–C(7a) 1.799(4), O(9)···Cl⁻ 3.042(4); C(3)–S(4)–C(5) 104.1(2), C(3)–S(4)–C(7a) 93.8(2), C(5)–S(4)–C(7a) 97.7(2), C(6)–S(7)–C(7a) 93.3(2).

chloride in pyridine at −10 °C for two days was exceptionally clean. TLC and ^{13}C NMR examination of the crude product mixture revealed that, along with unchanged starting material, the bicyclic sulfonium salt **8** is essentially the only reaction product formed. Although the polar product **8** decomposed on attempted silica gel column purification under Ganem's conditions (MeCN–H_2O–HOAc; 20 : 4 : 1),[15] it was obtained pure (40–70% recovery) using CH_2Cl_2–MeOH 10–100% as the eluting system (m.p. 176–180 °C decomp. (MeOH); $[\alpha]_D$ = −127.3; *c* = 0.94, H_2O).

A ^1H,^1H 2D COSY NMR experiment established that the resonance appearing furthest downfield [δ 5.38 (d), *J* 5.5 Hz] corresponded to H(7a) in analogue **8**, but the intermediate value of the coupling constant for this signal rendered tenuous the attribution of the relative stereochemistry for this centre. However, the observed correlations of H(2) [and H(1)] with H(6) in the 2D NOESY spectrum favour structure **8** over the alternative structure **8′** arising from displacement of OTs^- ion in conformer **7′**. This cyclization mode is also unexpected on steric grounds. In the ^{13}C NMR spectrum of **8** the observed *ca* 10 ppm down field shift of the C(5) (δ 51), and C(7a) (δ 69) absorptions, and the 4 ppm upfield shift of the C(6) signal (δ 36) relative to the corresponding signals in **6**, are in keeping with their position α- and β-respectively to positively charged sulfur.[16]

The *cis*-fused structure of compound **8** was finally confirmed by an X-ray diffraction study (Fig. 1).† The torsion angle values indicate that the dihydroxy substituted ring adopts an envelope form with C(2) out of the plane of the other four atoms by 0.647(3) Å, and that the other ring exits in a half chair conformation with atoms C(6) and S(7) out of plane by −0.373(4) and 0.529(4) Å, respectively. An ionic bond exists between the positive sulfur and a chloride ion located in the direction of the C(7a)–S(4) bond and in front of S(4) by 3.314 Å [C(7a)–S(4)···Cl^- angle = 171.5°]. Furthermore, each hydroxy group is hydrogen bonded: O(9)H with the nearest Cl^- ion [O(9)H···Cl^- 3.043(4), H_O(9)···Cl^- 2.05 Å and the angle O(9)–H···Cl^- = 173.8°] and O(10)H with the O(9)H of a neighbouring molecule [O(10)–H···O(9′) 2.806(4), H_O(10)···O(9) 1.83 Å and the angle O(10)–H···O(9′) 164.4°].

Sulfonium salt **8** has been tested at 1 mmol dm^{-3} concentration for its ability to inhibit twelve different human liver glycosidases.[5] The observation that it is stable in D_2O for periods exceeding one week at ambient temperatures and slowly decomposes in methanol solution only after extended periods suggests that the molecule would remain intact under the assay conditions. Analogue **8** is an inhibitor of α-mannosidases (76% at pH 6.5 and 48% at pH 4), and although somewhat poorer than swainsonine (100% at pH 4 and 95% at pH 6.5), it compares well with deoxymannonojirimicin (DMJ) (61% at pH 4 and 37% at pH 6.5) and is considerably better than the ring contracted analogues **3a,b** which inhibit the lysosomal and neutral enzymes between 8 and 50%.[7] However, in contrast to swainsonine and DMJ, salt **8** shows good selectivity, only inhibiting appreciably α-mannosidase and α-fucosidase (41% at pH 5.5). To clarify the effect of pH on inhibition, the lysosomal and neutral α-mannosidases with acidic (pH 4) and neutral (pH 6.5) optima, respectively, were separated by chromatography on concanavalin A-Sepharose and retested.[5] Compound **8** has no effect on the neutral α-mannosidase which implies that increased inhibition of the crude α-mannosidase at pH 6.5 was due to a greater inhibition of lysosomal isoenzyme at higher pH's. Interestingly, lysosomal α-mannosidase shows a similar pH dependence of inhibition by amino sugars such as swainsonine.[5] It is also interesting that although 8a-episwainsonine, 8,8a-diepiswainsonine and the pyrrolidines 1,4-dideoxy-1,4-imino-D-talitol and 1,4-dideoxy-1,4-imino-L-allitol, all of which possess the same stereochemistry at centres 1, 2 and 7a of compound **8**, are better inhibitors than **8**,[5] they discriminate only partially between neutral and lysosomal α-mannosidases. In contrast,

† *Crystal data*: $[C_6H_{11}O_2S_2]^+Cl^-$, M_w = 214.73, colourless crystal: 0.10 × 0.25 × 0.50 mm, orthorhombic, space group $P2_12_12_1$ (no. 19), *a* = 6.226(3), *b* = 11.247(6), *c* = 12.780(7) Å, *V* = 894.9(8) $Å^3$, *Z* = 4, D_c = 1.594 g cm^{-3}, *F*(000) = 448, λ (Cu-Kα) = 1.5418 Å, μ = 7.54 mm^{-1} (absorption ignored).

Data were measured on an Enraf-Nonius CAD4 diffractometer, using graphite monochromated Cu-Kα radiation, and the θ–2θ scan technique up to θ = 68°. A total of 1904 data were collected, of which 1598 were unique (−7 ≤ *h* ≤ 7, *k*, 0–13, *l*, 0–15). Cell parameters were obtained from 25 well centred reflections with 7.0 ≤ θ ≤ 23.3°. The structure was solved by direct methods (SHELXS86[17]) and refined by full-matrix least-squares analysis (SHELX76[18]) to a *R* value of 0.046, R_w = 0.058 (with $w = 1/\sigma^2(F_o) + 0.0029\ F_o^2$) for the 1526 observed reflections having $I \geq 3\sigma(I)$. All the hydrogen atoms were located on a difference Fourier map, and introduced in the refinement at theoretical positions (dC–H or O–H = 1.00 Å) with an isotropic thermal factor equivalent to that of the bonded atom (+10%). The absolute configuration was confirmed by a comparison of the Bijvoët difference pairs.

Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

the pyrrolizidine analogue **8** specifically inhibits the lysosomal enzyme.

Received, 22nd July 1992; Com. 2/03936B

References

1 R. J. Molyneux, M. Benson, R. Y. Wong, J. E. Tropea and A. D. Elbein, *J. Nat. Prod.*, 1988, **51**, 1198.
2 R. J. Nash, L. E. Fellows, J. V. Dring, G. W. J. Fleet, A. E. Derome, T. A. Hamor, A. M. Scofield and D. J. Watkin, *Tetrahedron Lett.*, 1988, **29**, 2487.
3 Naturally occurring australine epimers include: 3-epiaustraline: R. J. Nash, L. E. Fellows, A. C. C. Plant, G. W. J. Fleet, A. E. Derome, P. D. Baird, M. P. Hegarty and A. M. Scofield, *Tetrahedron*, 1988, **44**, 5959; 1-epiaustraline: C. M. Harris, T. M. Harris, R. J. Molyneux, J. E. Tropea and A. D. Elbein, *Tetrahedron Lett.*, 1989, **30**, 5685; 7-epiaustraline: R. J. Nash, L. E. Fellows, J. V. Dring, G. W. J. Fleet, A. Girdhar, N. G. Ramsden, J. M. Peach, M. P. Hegarty and A. M. Scofield, *Phytochemistry*, 1990, **29**, 111.
4 N. M. Carpenter, G. W. J. Fleet, I. Cenci di Bello, B. Winchester, L. E. Fellows and R. J. Nash, *Tetrahedron Lett.*, 1989, **30**, 7261; K. Burgess and I. Henderson, *Tetrahedron Lett.*, 1990, **31**, 6949.
5 I. Cenci di Bello, G. Fleet, S. K. Namgoong, K.-I. Tadano and B. Winchester, *Biochem. J.*, 1989, **259**, 855.
6 G. W. J. Fleet, P. W. Smith, S. V. Evans and L. E. Fellows, *J. Chem. Soc. Chem. Commun.*, 1984, 1240.
7 A. J. Fairbanks, G. W. J. Fleet, A. H. Jones, I. Bruce, S. A. Daher, I. Cenci di Bello and B. Winchester, *Tetrahedron*, 1991, **47**, 131.
8 J. E. Tropea, R. J. Molyneux, G. P. Kaushal, Y. T. Pan, M. Mitchell and A. D. Elbein, *Biochemistry*, 1989, **28**, 2027.
9 P. R. Dorling, C. R. Huxtable and S. M. Colegate, *Biochem. J.*, 1980, **191**, 649.
10 F. J. Urban, R. Breitenbach and L. A. Vincent, *J. Org. Chem.*, 1990, **55**, 3670; H. Matsuyama, T. Nakamura and N. Kamigata, *J. Org. Chem.*, 1989, **54**, 5218; see also: K. Tani, S. Otsuka, M. Kido and I. Miura, *J. Am. Chem. Soc.*, 1980, **102**, 7396.
11 D. Seebach, N. R. Jones and E. J. Corey, *J. Org. Chem.*, 1967, **33**, 300; E. L. Eliel, R. O. Hutchins, R. Mebane and R. L. Willer, *J. Org. Chem.*, 1976, **41**, 1052; D. M. Roush, E. M. Price, L. K. Templeton, D. H. Templeton and C. H. Heathcock, *J. Am. Chem. Soc.*, 1979, **101**, 2971; A. Taylor, A. J. Blake, A. J. Holder, T. I. Hyde and M. Schroder, *New J. Chem.*, 1991, **15**, 511.
12 N. A. Hughes and R. Robson, *J. Chem. Soc. C*, 1966, 2366; K. Blumberg, A. Fucello and T. van Es, *Carbohydr. Res.*, 1979, **70**, 217; E. T. Jarvi and B. W. Metcalf, *Carbohydr. Res.*, 1989, **187**, 159.
13 B. Classon, P. J. Garegg, B. Samelsson and Z. Liu, *J. Carbohydr. Chem.*, 1987, **6**, 593; M. W. Bredenkamp, C. W. Holzapfel and A. D. Swanepoel, *Tetrahedron Lett.*, 1990, **31**, 2759; M. R. Dyson, P. L. Coe and R. T. Walker, *Carbohydr. Res.*, 1991, **216**, 237.
14 For a pertinent example related directly to the present work, see: J. Theim and H.-P. Wessel, *Analen, Chem.*, 1982, 607.
15 M. K. Tong, G. Papandreou and B. Ganem, *J. Am. Chem. Soc.*, 1990, **112**, 6137.
16 J. B. Lambert, D. A. Netzel, H. Sun and K. K. Lilianstrom, *J. Am. Chem. Soc.*, 1976, **98**, 3778; G. Barbarella and P. Dembech, *Org. Magn. Reson.*, 1981, **15**, 72.
17 G. M. Sheldrick, SHELXS86, 1986, program for crystal structure determination, University of Göttingen, Germany.
18 G. M. Sheldrick, SHELX76, 1976, program for crystal structure determination, University of Cambridge, England.