Aust. J. Chem., 1984, **37**, 1503–9

The Synthesis and Biological Activity of (±)-(1α,2α,8aα)-Indolizidine-1,2-diol

Steven M. Colegate, Peter R. Dorling and Clive R. Huxtable

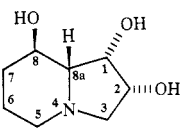
School of Veterinary Studies, Murdoch University, Murdoch, W.A. 6150.

Abstract

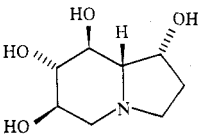
The racemic indolizidine-1,2-diol (6) was synthesized and found to be a weak, *in vitro* inhibitor of acid α-D-mannosidase (K_m $0{\cdot}75 \times 10^{-2}$ M) and acid α-D-glucosidase (K_m $1{\cdot}1 \times 10^{-2}$ M).

Introduction

A naturally occurring indolizidinetriol (swainsonine) (1), isolated from the plants *Swainsona canescens*[1] and *Astragalus lentiginosus*[2] and from the fungus *Rhizoctonia leguminicola*,[3] has been shown to be a potent, reversible, active-site directed inhibitor of lysosomal α-mannosidase.[4]



(1)



(2)

Further to this, another naturally occurring hydroxylated indolizidine, castanospermine (2),[5] has been shown to inhibit β-glucosidases and β-glucocerebrosidase.[6]

These occurrences suggest a new class of enzyme inhibitors based upon the indolizidine skeleton and differing in the degree, position and stereochemistry of hydroxylation.

It has been suggested[3,4,7] that the indolizidinetriol swainsonine (1) owes its activity and specificity to the similarity of the position and orientation of the hydroxy substituents on the swainsonine cation to those of the mannosyl cation proposed as an intermediate in the enzymatic hydrolysis of mannopyranosides.[8–10]

[1] Colegate, S. M., Dorling, P. R., and Huxtable, C. R., *Aust. J. Chem.*, 1979, **32**, 2257.
[2] Molyneux, R. J., and James, L. F., *Science*, 1982, **216**, 190.
[3] Schneider, M. J., Ungemach, F. S., Broquist, H. P., and Harris, T. M., *Tetrahedron*, 1983, **39**, 29.
[4] Dorling, P. R., Huxtable, C. R., and Colegate, S. M., *Biochem. J.*, 1980, **191**, 649.
[5] Hohenschutz, L. D., Bell, E. A., Jewess, P. J., Leworthy, O. P., Pryce, R. J., Arnold, E., and Clardy, J., *Phytochemistry*, 1981, **20**, 811.
[6] Saul, R., Chambers, J. P., Molyneux, R. J., and Elbein, A. D., *Arch. Biochem. Biophys.*, 1983, **221**, 593.
[7] Dorling, P. R., Colegate, S. M., and Huxtable, C. R., *Toxicon*, 1983, Suppl. No. 3, 93.
[8] Leaback, D. H., *Biochem. Biophys. Res. Commun.*, 1968, **32**, 1025.
[9] Levvy, G. A., and Snaith, S. M., *Adv. Enzymol. Relat. Areas Mol. Biol.*, 1972, **36**, 151.
[10] De Prijcker, J., and De Bruyne, C. K., *Carbohydr. Res.*, 1975, **43**, 173.

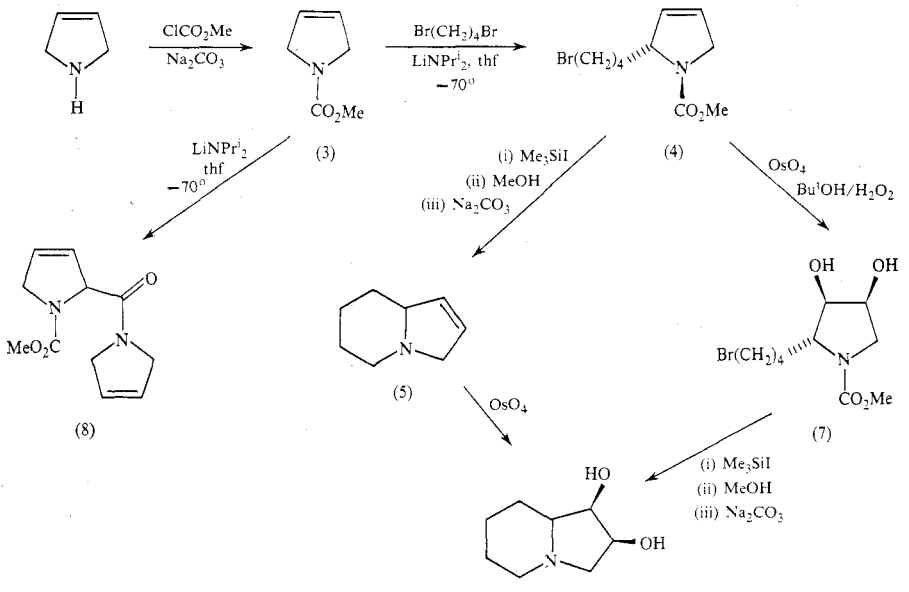
0004-9425/84/071503$02.00

If this is the situation then it may be feasible to synthesize specific inhibitors of other enzymes by altering the degree, position and stereochemistry of substitution by hydroxy groups into the indolizidine skeleton.

The title compound was thus synthesized in order to determine any biological activity. Indeed, it proved to be a weak inhibitor of acid α-D-mannosidase and a weak inhibitor of acid α-D-glucosidase compared to the effect of swainsonine and castanospermine, at similar concentrations, on these enzymes.

Discussion

The approach to the *cis*-indolizidinediol involved α-alkylation of the *N*-protected 3-pyrroline (3) as shown in Scheme 1. The α-alkylation of the anion generated from the pyrroline urethane has been successfully used by Armande[11] in the synthesis of the 12-azaprostanoid skeleton. Also, α-alkylation with a halogenated compound followed by hydrolysis of the urethane and subsequent cyclization affords the dehydroindolizidine system as demonstrated by Macdonald in the synthesis of the pharaoh ant trail pheromone.[12]



Scheme 1

3-Pyrroline was prepared by zinc/hydrochloric acid reduction of pyrrole and could be separated from the fully reduced pyrrolidine either by fractional recrystallization of the hydrochloride salts[13] or by fractional recrystallization of the subsequent urethanes from ether/pentane solutions.

The alkylation was effected by treating the 3-pyrroline urethane with lithium diisopropylamide ($LiNPr^i_2$) in the presence of 1,4-dibromobutane in dry tetrahydrofuran at −70°. The anion generated from the urethane (3), in the presence of $LiNPr^i_2$,

[11] Armande, J. C., and Pandit, U. K., *Tetrahedron Lett.*, 1977, 897.
[12] Macdonald, T. K., *J. Org. Chem.*, 1980, **45**, 193.
[13] Hudson, C. B., and Robertson, A. V., *Tetrahedron Lett.*, 1967, **41**, 4015.

is very reactive and thus quickly reacts with another molecule of the urethane, forming the dimer (8), unless alkylation is effected immediately the anion is formed.[14]

Repeated flash chromatography affords the racemic α-alkylated compound (4) which is then treated with a catalytic amount of osmium tetroxide in t-butyl alcohol/hydrogen peroxide (Milas reagent) to produce the *cis*-diol (7).

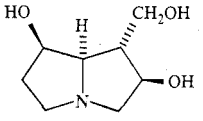
Further reaction with trimethylsilyl iodide followed by methanolysis of the resulting trimethylsilyl carbamate[15] and cyclization afforded the indolizidinediol (6).

An alternative route involved converting the carbamate (4) into the corresponding amine followed by cyclization to the 3,5,6,7,8,8a-hexahydroindolizine (5). However, subsequent glycolation of (5) was only effective when stoichiometric amounts of OsO_4 were used, and the yields were very low, possibly due to complexation of the osmium tetroxide by the nitrogen atom in (5). However, the diols obtained by either route were identical in their spectroscopic and physical properties and in their biological activity.

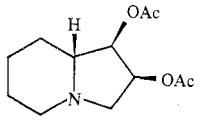
The indolizidinediol (6) (and its diacetate) were characterized by mass spectroscopy, i.r. spectroscopy and by ^1H and ^{13}C n.m.r. spectroscopy.

That the ring junction is *trans* is demonstrated by the presence of Bohlmann bands in the i.r. spectra of both the diol (6) and the unsaturated compound (5). These characteristic Bohlmann bands (2700–2850 cm^{-1}) are observed when two or more α-hydrogens are *trans*-diaxial to the lone pair on nitrogen.[16,17]

If, as in the case of swainsonine diacetate,[1] the indolizidinediol (6) molecule is oriented so that the lone pair of electrons on nitrogen are below the plane of the ring, then H 8a must be β. Analysis of the 80-MHz ^1H n.m.r. spectrum of the diacetate of (6), in conjunction with reference to molecular models and similar compounds, indicates that the protons H 1 and H 2 are *cis* to each other but *trans* with respect to H 8a and therefore are α. The *trans* relationship of H 8a and H 1 is reflected in the large coupling constant observed ($J_{8a,1}$ 8·2 Hz) and is supported by the observation that $J_{7a,1}$ of the pyrrolizidine alkaloid croalbinecine (9) which has the same relative configuration, is of the same magnitude ($J_{7a,1}$ 8 Hz).[18]



(9)



(10)

Thus the relative configuration of the indolizidinediol (6) and its diacetate (10), when described with respect to swainsonine, is predicted to be 1β,2β,8aβ. However, for registry purposes, the racemic compound should be described as 1α,2α,8aα.

This predicted relative configuration is in accord with the supposition that the α-alkylated 3-pyrroline urethane (4) is *cis*-glycolated on the least hindered side to afford the diol (7) in which H 2 (which becomes H 8a of the indolizidinediol) and H 3 (which becomes H 1 of the indolizidinediol) are *trans* to each other.

[14] Macdonald, T. L., personal communication.
[15] Jung, M. E., and Lyster, M. A., *J. Chem. Soc., Chem. Commun.*, 1978, 315.
[16] Luning, B., and Lundin, C., *Acta Chem. Scand.*, 1967, **21**, 2136.
[17] Rader, C. P., Young, R. L., and Aaron, H. S., *J. Org. Chem.*, 1965, **30**, 1563.
[18] Sawhney, R. S., Atal, C. K., Culvenor, C. C. J., and Smith, L. W., *Aust. J. Chem.*, 1974, **27**, 1805.

The *cis*-diol (6) was tested for biological activity as described by Dorling[4] by incubating it in the presence of a liver homogenate and the appropriate umbelliferyl substrates.

The indolizidinediol racemate was found to weakly inhibit both acid α-mannosidase and acid α-glucosidase. The concentrations of diol required to effect 50% inhibition (K_m) were approximately $0\cdot75\times10^{-2}$ M and $1\cdot1\times10^{-2}$ M respectively. Swainsonine, at a concentration of $6\cdot3\times10^{-2}$ M, had no effect on the activity of acid α-glucosidase and completely inhibited the activity of acid α-mannosidase as expected. Castanospermine, at a similar concentration, had no effect on the activity of acid α-mannosidase but completely inhibited acid α-glucosidase activity.

Neither swainsonine, castanospermine nor the indolizidinediol had any effect at these concentrations on 2-hexosaminidase activity.

The greatly decreased activity of the diol (6) as an inhibitor of acid α-mannosidase (K_m $0\cdot75\times10^{-2}$ M), compared to swainsonine (K_m 2×10^{-8} M),[4] may be attributed to a less rigid spatial similarity to the postulated mannosyl cation involved in the enzymatic hydrolysis. It is probable that C 1–OH, C 2–OH, C 3 and N are essential for recognition by α-mannosidase and that the C 8–OH, in the case of swainsonine, serves to enhance the spatial similarity, perhaps by intramolecular hydrogen bonding, thus increasing the affinity of the active enzyme site for swainsonine and increasing the specificity of swainsonine for α-mannosidase. As the spatial restraints are eased slightly as in the case of the indolizidinediol (6), then the compound demonstrates less affinity for the enzyme and its activity is less specific to the extent that the compound is recognized by both a mannose substrate site and a glucose substrate site to approximately the same degree.

Experimental

Melting points were determined in an Electrothermal melting point apparatus and are uncorrected. The 90-MHz ^{1}H and 20·1-MHz ^{13}C n.m.r. spectra (of solutions in $CDCl_3$) and low-resolution mass spectra were recorded at the Chemistry Department of the University of Western Australia by using a Brüker XH-90, a Brüker WP-80 and a Hewlett–Packard 5986 g.c./mass spectrometer respectively. I.r. spectra (of chloroform solutions in KBr cells) were recorded in a Perkin–Elmer 282 spectrophotometer. When necessary, the differentiation of quaternary carbons or secondary carbons from tertiary or primary carbons in the ^{13}C n.m.r. spectra, was done by a gated spin echo experiment.[19,20] Chemical shifts (δ) are given with respect to the chemical shift of $SiMe_4$. Assignments are made with due regard to the additivity rules, multiplicity and comparison with the spectra of similar compounds. Kieselgel 60G (Merck) and Kieselgel 60 (Merck) were used as the adsorbents for t.l.c. and flash column chromatography[21] respectively.

Preparation of 3-Pyrroline

This was prepared by the method of Andrews[22] and purified, when necessary, by repeated recrystallizations of the hydrochloride salts from ethanol.[13] ^{1}H n.m.r. δ 4·05, 4H, s; 5·74, 2H, s, olefinic; 7·7, 1H, broad s, NH. ^{13}C n.m.r. δ 59·4, t, NCH_2; 127·7, d, vinylic.

Preparation of Methyl 3-Pyrroline-1-carboxylate (3)

3-Pyrroline (4·74 g, 0·059 mol) was dissolved in ethanol (30 ml) and stirred with sodium bicarbonate. Methyl chloroformate (6 ml, 7·32 g, 0·077 mol) was then added cautiously and the mixture

[19] Le Cocq, C., and Lallemand, J.-Y., *J. Chem. Soc., Chem. Commun.*, 1981, 150.
[20] Cookson, D. J., and Smith, B. E., *Org. Magn. Reson.*, 1981, **16**, 111.
[21] Still, W. C., Khan, M., and Mitra, A., *J. Org. Chem.*, 1978, **43**, 2923.
[22] Andrews, L. H., and McElvain, S. M., *J. Am. Chem. Soc.*, 1929, **51**, 889.

stirred at room temperature for 2 h. The reaction mixture was then filtered, concentrated under vacuum and the residue distributed between ether and water.

The ether layer was dried (Na_2SO_4) and evaporated to afford a colourless liquid. Distillation under vacuum afforded a colourless liquid which rapidly crystallized forming a white solid (5·08 g, 0·04 mol), m.p. 41–42°, 58% yield. G.c.–m.s., OV-101 column, 0·31 mm by 25 mm WCOT capillary, temperature program 40° to 120° during 5 min. Flowrate 7·5 ml/min; R_t 3·9 min, *m/e* 127 (M), 3-pyrroline urethane; R_t 4·1 min, *m/e* 129 (M), pyrrolidine urethane. ^{1}H n.m.r. δ 5·8, 2H, broad multiplet, vinylic; 4·16, 4H, broad doublet, J 1·7 Hz, methylene; 3·71, 3H, s, methoxy. ^{13}C n.m.r. δ 155·43, s, carbonyl; 126·11, d, olefinic C3 and C4; 53·63, t, C2 or C5; 53·12, t, C5 or C2; 52·38, q, OCH_3. *m/e* 127 (M, 21%), 112 (100), 96 (5), 68 (45).

α-Alkylation of the Pyrroline Urethane (3)

A solution of the pyrroline urethane (3) (1·5 g, 0·012 mol) and 1,4-dibromobutane (5 ml, 9·1 g, 0·04 mol) in tetrahydrofuran (100 ml, dried by distillation from sodium hydride) was stirred under nitrogen at −70°C. To this was added small, successive aliquots of a freshly prepared solution of $LiNPr^i_2$ in dry tetrahydrofuran at −70°.

The progress of the reaction was monitored by t.l.c. (light petroleum/ethyl acetate 4 : 1).

When reaction was complete the reaction mixture was allowed to warm to room temperature and the solvent evaporated under vacuum. The yellow/orange residue was distributed between 0·1 N aqueous HCl and ether. The ether layer was washed with water, dried and evaporated to afford a pale yellow liquid.

Repeated flash chromatography on Kieselgel 60 with light petroleum/ethyl acetate mixtures as the eluent afforded the racemic product (4) as a colourless liquid (2·17 g, 8×10^{-3} mol, 70% yield) which decomposed when heated to boiling point at atmospheric pressure. ^{13}C n.m.r. δ 155·4, s, carbonyl; 129·9, d, vinylic; 64·5, d, NCH; 53·7, t, methylene NCH_2; 52·2, q, methoxy; 33·4, t, $(CH_2)_3CH_2Br$; 23·0, t, $(CH_2)_3CH_2Br$. Mass spectrum, *m/e* 182 (M−Br; 1%), 126 (100).

Dimerization of the Pyrroline Urethane (3)

The pyrroline urethane (3) was treated with $LiNPr^i_2$ in dry tetrahydrofuran as described previously except that 1,4-dibromobutane was not added. The reaction mixture, now dark brown in colour, was worked up as before to afford a pale yellow solid. Recrystallization from chloroform/ether afforded a white solid, m.p. 136–138° (dec.), 65% yield. ^{13}C n.m.r. δ 155·1, s, carbonyl; 154·8, s, carbonyl; 129·3, d, vinylic; 126·1, d, vinylic; 125·1, d, vinylic; 124·6, d, vinylic; 65·9, d, NCH; 54·3, t, NCH_2; 53·6, t, NCH_2; 52·9, t, NCH_2; 52·6, q, OCH_3. *m/e* 222 (M, 2%), 126 (100), 96 (4).

cis-*Glycolation of Methyl 2-(4-Bromobutyl)-3-pyrroline-1-carboxylate (4)*

To a stirred solution of the urethane (4) (0·70 g, 2·6 mol) in Bu^tOH/H_2O_2 (20 ml of a solution prepared by mixing and drying Bu^tOH (100 ml) and H_2O_2 (25 ml 30% solution)) at 0°, was added a solution of OsO_4 (2 ml of a 1% solution in Bu^tOH).

The yellow reaction mixture was stirred at room temperature for 6 h during which time it became colourless and t.l.c. ($CHCl_3$/MeOH 4 : 1) indicated complete reaction of the starting material. The reaction mixture was then diluted with water and concentrated under vacuum. The aqueous residue was extracted with $CHCl_3$.

The $CHCl_3$ extract was dried (Na_2SO_4) and evaporated to afford a colourless liquid. Flash chromatography of the product on silica gel, with $CHCl_3$/MeOH (4 : 1) as the eluent, afforded the required diol (7) as a colourless liquid (0·7 g, 2·3 mmol, 91% yield). ^{13}C n.m.r. δ 156·5, s, carbonyl; 75·7, d, 3-OH; 69·9, d, 4-OH; 63·3, d, C2; 52·7, q, methoxy; 50·5, t, C5; 33·6, t, $CH_2(CH_2)_3Br$; 32·4, t, $CH_2CH_2(CH_2)_2Br$; 31·2, t, $(CH_2)_2CH_2CH_2Br$; 24·1, t, $(CH_2)_3CH_2Br$. I.r. 3440s (H-bonded OH), 1690s cm^{-1} (urethane carbonyl). *m/e* 266/264 (M−OCH_3, 1%), 216 (32), 160 (100), 142 (30).

Preparation of the Indolizidinediol (6)

To a stirred solution of methyl 2-(4-bromobutyl)-3,4-dihydroxypyrrolidine-1-carboxylate (7) (0·45 g, 1·5 mmol) in dry benzene (10 ml) at 50° was added a solution of trimethylsilyl iodide

(1·6 mmol) (prepared by the method used by Jung[23]) in dry benzene (10 ml). The resultant red solution was heated at 50° for 1 h, the progress of reaction being monitored by t.l.c. ($CHCl_3$/MeOH, 4 : 1).

The reaction mixture was cautiously diluted with methanol and then concentrated under vacuum. The red, liquid residue was dissolved in methanol (20 ml) and stirred in the presence of Na_2CO_3 for 1 h; the colour disappeared.

The reaction mixture was filtered and acidified with 1 N HCl. The resulting acid solution was applied to a cation exchange resin column (Amberlite IR-120, NH_4^+), washed with water and then eluted with 2% aqueous ammonia.

The basic fractions containing the product were identified by t.l.c., combined and freeze-dried to afford a yellow powder.

This residue was extracted with $CHCl_3$ which was subsequently dried and evaporated to afford a pale yellow liquid (0·21 g).

Flash chromatography of this product on silica gel with $CHCl_3$/MeOH (19 : 1) as the eluent afforded the *indolizidinediol* (6) as a colourless liquid (0·106 g, $6·8 \times 10^{-4}$ mol), 45% yield (Found: $M^{+\cdot}$, 157·1105. $C_8H_{15}NO_2$ requires $M^{+\cdot}$, 157·1103). ^{13}C n.m.r. δ, 74·4, d, C8a; 67·9, d, C1; 67·1, d, C2; 61·3, t, C3; 52·8, t, C5; 27·9, t, C8; 24·5, t, C6; 23·5, t, C7. ^1H n.m.r. δ 4·0–4·4, multiplet, H1 and H2; 3·65, dd, $J_{3d,2}$ 8 Hz, $J_{3d,3u}$ 14 Hz, H3d; 3·11, broad doublet, $J_{5d,5u}$ 9 Hz, H5d; 2·6–1·00, superimposed multiplets, H3u, H5u, H6, H7, H8, H8a. *m/e* 157 (M, 6%), 156 (4), 97 (100), 96 (22). I.r. 3400s (H-bonded OH), 2860m, 2810m, 2740mw (Bohlmann bands for *trans*-fused ring system).

Preparation of the Diacetate (10)

A solution of the diol (6) (80 mg, $5·1 \times 10^{-4}$ mol) in chloroform (10 ml) was stirred for 16 h with acetic anhydride (3 ml). The reaction mixture was diluted with water and concentrated under vacuum. The aqueous residue was made basic (Na_2CO_3) and extracted with chloroform. The pale yellow chloroform solution was then extracted with 0·1 N HCl which removed the desired product from the coloured $CHCl_3$ solution. The aqueous acid solution was made basic (Na_2CO_3) and extracted with $CHCl_3$. Drying and evaporation of the chloroform extract afforded the required diacetate as a colourless liquid which rapidly crystallized (80 mg, 73% yield), m.p. 41–42°.

Further purification could be attained, if necessary, by flash chromatography on silica gel with $CHCl_3$/MeOH (19 : 1) as the eluent. ^{13}C n.m.r. δ 170·1, s, carbonyl; 75·3, d, C8a; 68·6, d, C1; 65·2, d, C2; 59·6, t, C3; 52·8, t, C5; 28·8, t, C8; 25·2, t, C6; 23·7, t, C7; 20·7, q, acetoxy CH_3; 20·5, q, acetoxy CH_3. ^1H n.m.r. δ 5·22, 1H, ddd, $J_{2,1}$ 8·2 Hz, $J_{2,3d}$ 6·9 Hz, $J_{2,3u}$ 5 Hz, H2; 4·74, 1H, dd, $J_{1,2}$ 8·2 Hz, $J_{1,8a}$ 8·2 Hz, H1; 3·58, 1H, dd, $J_{3d,2}$ 6·9 Hz, $J_{3d,3u}$ 10 Hz, H3d; 3·02, 1H, broad d, $J_{5d,5u}$ 10 Hz, H5d; 2·06, acetoxy CH_3; 2·04, acetoxy CH_3; 2·5–1·09, superimposed multiplets, H3u, H5u, H6, H7, H8, H8a. *m/e* 241 (M, 1%), 182 (9), 181 (5), 122 (100).

Preparation of 3,5,6,7,8,8a-Hexahydroindolizine (5)

The α-alkylated pyrroline urethane (4) was treated with trimethylsilyl iodide, methanol and Na_2CO_3 as previously described. The colourless methanolic solution was acidified and concentrated under reduced pressure. The residual aqueous, acidic solution was extracted with chloroform. The aqueous layer was then made basic and reextracted with chloroform. The dried chloroform layer was evaporated to afford a colourless liquid (76% yield), b.p. 83–84° ^{13}C n.m.r. δ 133·8, d, C1 vinylic; 128·2, d, C2 vinylic; 67·4, d, C8a; 57·6, t, C3; 50·0, t, C5; 29·6, t, C8; 24·9, t, C6; 24·2, t, C7. ^1H n.m.r. δ 5·73, 2H, broadened s, vinylic; 2·7–3·8, 4H, superimposed multiplets, H3, H5; 2·2–2·7, 1H, multiplet, H8a; 1·0–2·1, 6H, superimposed multiplets, H6, H7, H8. *m/e* 123 (M, 50%), 122 (55), 95 (8), 81 (100). I.r. 2880s, 2855s, 2790m, 2695wm (Bohlmann bands indicating *trans*-fused ring system), 1610w cm^{-1} (symmetrical alkene).

Acknowledgments

The authors would like to thank Dr L. Byrne for recording the ^1H and ^{13}C n.m.r. spectra and Dr T. Macdonald of Vanderbilt University for his helpful suggestions

[23] Jung, M. E., and Lyster, M. A., *J. Am. Chem. Soc.*, 1977, **99**, 968.

concerning the α-alkylation of the 3-pyrroline urethane. The sample of castanospermine was graciously supplied by Professor E. A. Bell of Jodrell Laboratory, Kew. The above work was funded by a grant from the Australian Research Grants Committee.

Manuscript received 9 November 1983