Phytochemistry, Vol 29, No 1, pp 111–114, 1990
Printed in Great Britain

0031 9422/90 $3.00 + 0.00
Pergamon Press plc

TWO ALEXINES [3-HYDROXYMETHYL-1,2,7-TRIHYDROXYPYRROLIZIDINES] FROM *CASTANOSPERMUM AUSTRALE*

ROBERT J. NASH, LINDA E. FELLOWS, JANET V DRING, GEORGE W J. FLEET,* AARTI GIRDHAR,† NIGEL G. RAMSDEN,* JOSEPHINE M. PEACH,* MERVYN P HEGARTY‡ and ANTHONY M. SCOFIELD§

Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, U.K., *Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY, U.K., †Chemical Crystallography Laboratory, 9 Parks Road, Oxford OX1 3PD, U.K., ‡Cunningham Laboratory, CSIRO, St Lucia, Queensland 4067, Australia, §Department of Biochemistry and Biological Sciences, University of London, Wye College, Ashford, Kent TN25 5AH, U.K.

(*Received* 26 *July* 1989)

Key Word Index—*Castanospermum australe*, Leguminosae, alexines, glycosidase inhibition, pyrrolizidine, amyloglucosidase, alkaloids

Abstract—Two new alexines have been isolated from *Castanospermum australe* The structure of 1,7a-diepialexine was firmly established by X-ray crystallographic analysis of the corresponding 1,7-isopropylidene derivative The structure of the second new alexine was tentatively assigned as 7,7a-diepialexine or its enantiomer. The abilities of naturally occurring alexines to inhibit mouse gut disaccharidase and fungal glucan 1,4-α-glucosidase are compared

INTRODUCTION

Alexine (**1**), the first example of a pyrrolizidine alkaloid with a carbon branch at C-3, was isolated in 1987 [1] from *Alexa leiopetala*, a leguminous tree from the forests of Guyana, Surinam, French Guiana, Venezuela and the Amazon basin. Subsequently, 3,7a-diepialexine (**2**) [2] and 7a-epialexine (australine) (**3**) [3] were isolated from *Castanospermum australe*, a rain forest tree from Queensland. Both the species contain the indolizidine glycosidase inhibitor castanospermine (**4**), and the genera are believed to be related, despite their geographical separation [4, 5]. This paper reports the isolation from *C australe of* two further diastereomers of alexine, and compares the ability of all the known naturally occurring alexines to inhibit mouse gut digestive glucosidases, and to inhibit fungal glucan 1,4-α-glucosidase.

RESULTS AND DISCUSSION

Structure determination

Both the new alexines had proton and carbon NMR spectra consistent with the structure of a 3-hydroxymethylpyrrolizidine; the NMR spectra of the two alkaloids were clearly different from the known naturally occurring alexines (**1–3**) and from two synthetic alexines, 3-epialexine (**5**) and 7-epialexine (**6**) [6]. Additionally, a characteristic fragmentation $[M+H-CH_2OH]^+$ in the mass spectra of the compounds strongly indicated a hydroxymethyl group attached to a carbon to the ring nitrogen. The structure of 1,7a-diepialexine [(1*S*,2*R*,3*R*,7*R*,7a*S*)-3-hydroxymethyl-1,2,7-trihydroxypyrrolizidine] (**7**) was firmly established by X-ray crystallographic analysis of the hydrochloride of 1,7-isopropylidene-(1*S*,2*R*,3*R*,7*R*,7a*S*)-3-hydroxymethyl-1,2,7-trihydroxypyrrolizidine (**8**); the structure of the other new alexine was tentatively assigned by spectroscopic techniques as 7,7a-diepialexine [(1*R*,2*R*,3*R*,7*R*,7a*R*)-3-hydroxymethyl-1,2,7-trihydroxypyrrolizidine] (**9**) or its enantiomer 1,2,3-triepialexine [(1*S*,2*S*,3*S*,7*S*,7a*S*)-3-hydroxymethyl-1,2,7-trihydroxypyrrolizidine] (**10**)

Glucosidase inhibition

Many polyhydroxylated alkaloids are potent and specific inhibitors of glycosidases in a range of organisms [8, 9]. Australine (**3**) has been shown to inhibit fungal glucan 1,4-α-glucosidase (amyloglucosidase) and glycoprotein processing glucosidase I [10] We have determined the activity of all the naturally occurring alexines against glucosidase (disaccharidase) activity in the mouse (Table 1) and against fungal glucan 1,4-α-glucosidase (amyloglucosidase) (Table 2) Despite some activity against α-glucosidase (**7** and **9**) and trehalase (**1**), these compounds appear to be weak inhibitors of mammalian digestive glycosidases compared to **4**, in contrast, all the compounds are strong inhibitors of the fungal glucan 1,4-α-glucosidase. Further evaluations of the biological activity of alexines are in progress During the course of this study, **9** was also isolated from *Alexa leiopetala*. confirming the close relationship between the two genera

EXPERIMENTAL

Isolation Ground freeze-dried seed (200 g) of *Castanospermum australe* A. Cunn (Queensland Herbarium voucher no BRI AQ 426819-M P HEGARTY) was extracted with 75% aq EtOH (2 × 4 l) and the combined extracts concd under vacuum The alkaloids were purified by ion-exchange chromatography on Amberlite CG 120 (NH_4^+) by elution with aq NH_4OH Three unidentified compounds were eluted after castanospermine (**4**) One was crystallized and shown by comparison of ^1H and

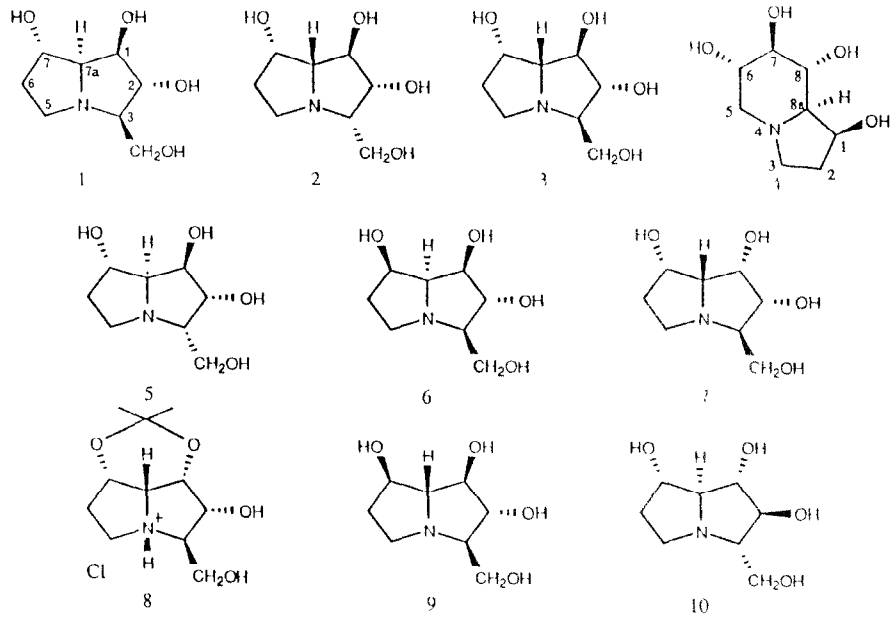

Table 1 Action of naturally occurring alexines on mouse gut digestive glucosidase (disaccharidase) activity compared with that of castanospermine

Inhibitor	*p*-Nitrophenyl α-D-glucopyranoside	*p*-Nitrophenyl β-D-glucopyranoside	Trehalose
1,7a-Diepialexine (**7**)	9.5×10^{-5}	NI	NI
7,7a-Diepialexine (**9**)	1.6×10^{-5}	2.3×10^{-4}	1.0×10^{-4}
Alexine (**1**)	NI	NI	5.9×10^{-5}
3,7a-Diepialexine (**2**)	NI	NI	NI
7a-Epialexine (**3**)	NI	3.3×10^{-4}	NI
Castanospermine (**4**)	2.8×10^{-6}	1.7×10^{-5}	9.8×10^{-6}

Concentration (M) of alkaloid giving 50% inhibition NI = less than 50% inhibition at 3.3×10^{-4}M

Table 2 Action of naturally occurring alexines on glucan 1,4-α-glucosidase-catalysed hydrolysis of potato amylose compared with that of castanospermine

Inhibitor	Concn (M) of alkaloid giving 50% inhibition
1,7a-Diepialexine (**7**)	1.5×10^{-6}
7,7a-Diepialexine (**9**)	1.3×10^{-5}
Alexine (**1**)	1.1×10^{-5}
3,7a-Diepialexine (**2**)	2.1×10^{-6}
7a-Epialexine (**3**)	1.5×10^{-6}
Castanospermine (**4**)	1.5×10^{-6}

^{13}C NMR data to be australine (**3**), [data for authentic australine being supplied by Dr R J Molyneux] The others, 1,7a-diepialexine (**7**) (130 mg) and 7,7a-diepialexine (**9**) (200 mg), were cored to oils and also converted to the corresponding hydrochlorides

Alkaloid peaks from the ion exchange column were analysed by gas chromatography of the pertrimethylsilyl derivatives on a 3% OV1 column (1.5 m × 4 mm) at 170 (isothermal) [11] The retention times of the trimethylsilylated derivatives relative to that of **4** were **7**, 0.62, **9** 0.62, **3**, 0.67 **2** 0.73 **1** 0.78

Spectral analysis ^{1}H NMR spectra in D_2O were run at 200 MHz on a Varian Gemini spectrometer or at 300 MHz on a Bruker WH 300 spectrometer or at 500 MHz on a Bruker WH 500 spectrometer ^{13}C NMR spectra in D_2O were recorded on a Bruker AM 250 (62.9 MHz). MeOH (δ 49.9) or dioxan (δ 67.3) were used as an internal standard Microanalyses were performed by the microanalytical services of the Dyson Perrins Laboratory

X-Ray crystal structure analysis The structure of the hydrochloride of 1,7-*O*-isopropylidene-1,7a-diepialexine[(1*S*,2*R*,3*R*,7*R*,7a*S*)-1,7-*O*-isopropylidene-3-hydroxymethyl-1,2,7-trihydroxypyrrolizidine] (**8**) was established by single crystal X-ray analysis The salt was recrystallized from Me_2CO-H_2O Cell dimensions and intensity data were measured with an Enraf-Nonius CAD4-F diffractometer up to ~75° (CuK_α radiation) The data were corrected for absorption Lorentz and polarization effects All calculations were carried out on a VAX 11 750 computer using SHELXS-86 [12] for direct methods and CRYSTALS [13] for all other calculations Atomic scattering factors were taken from International Tables [14] The coordinates of all non-hydrogen atoms were given by SHELXS-86 A difference map revealed the positions of the OH hydrogen atoms and all other hydrogen

atoms were placed geometrically. 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 merged Friedel pairs A correction for secondary extinction was applied and the model refined almost to convergence [15] The Flack enantiopole parameter [16] was refined for the data with unmerged Friedel pairs, its final value of 0 049 (e s d 0 004), together with calculation of Bijvoet pairs with the largest intensity differences indicated that the absolute configuration could be unambiguously determined from these X-ray data The merged data was refined with a Chebyshev weighting scheme [17] to give a final value of $R = 0\,035$ Atomic coordinates have been deposited at the Cambridge Crystallographic Data Centre, and are available on request, please quote full literature citation of the paper

1,7a-Diepialexine (**7**) Oil, $[\alpha]_D^{20} + 8\,5°$ (H_2O, *c* 0 41), ^{1}H NMR δ4 53 (*m*, 1H), 4 37 (*dd*, 1H), 3 91 (*dd*, 1H), 3 78 (*dd*, 1H), 3 60 (*m*, 2H), 3 22 (*m*, 1H), 3 13 (*m*, 1H), 3 07 (*m*, 1H), 2 01 (*m*, 2H) ^{13}C NMR· δ75 1 (*d*), 73 7 (*d*), 72 8 (*d*), 70 8 (*d*), 67 0 (*d*), 63.2 (*t*), 52 9 (*t*), 35 9 (*t*) MS *m/z* (DCI NH_3) 190 $[M+H]^+$ (56%), 158 $[M+H-CH_2OH]^+$ (100%)

1,7a-Diepialexine (**7**) *hydrochloride* $[\alpha]_D^{20} - 3\,0°$ (H_2O; *c* 0 4), ^{1}H NMR δ4 39 (*t*, 1H), 3 97 (*m*, 2H), 3 84 (*dd*, 1H), 3 67 (*dd*, 1H), 3 40 (*m*, 3H), 2 17 (*m*, 2H) ^{13}C NMR δ72 1 (*d*), 71 7 (*d*), 71 4 (*d*), 71 0 (*d*), 68 8 (*d*), 57 1 (*t*), 53 2 (*t*), 34 1 (*t*) MS *m/z* (DCI NH_3) 190 $[M+H]^+$ (42%), 158 $[M+H-CH_2OH]^+$ (100%) 1,7a-Diepialexine hydrochloride on crystallization from aq Me_2CO formed a crystalline acetonide (**8**), mp decomposes over 180°, $[\alpha]_D^{20} + 42\,2°$ (MeOH, *c* 0 27), ^{1}H NMR δ4 50 (*dd*, 1H), 4 23 (*dd*, 1H), 4 02 (*dd*, 1H), 3 89 (*dd*, 1H), 3 82 (*dd*, 1H), 3 74 (*dd*, 1H), 3 50 (*m*, 1H), 3 24 (*m*, 1H), 2 10 (*m*, 2H), 1 41 (*s*, 3H), 1 30 (*s*, 3H) ^{13}C NMR δ108 7 (*s*), 80 0 (*d*), 77 0 (*d*), 75 0 (*d*), 74 0 (*d*), 69.6 (*d*), 63 3 (*t*), 61 4 (*t*), 42 0 (*t*), 34 8 (*q*), 26 3 (*q*) MS *m/z* (DCI NH_3) 230 $[M+H]^+$ (100%), 198 $[M+H-CH_2OH]^+$ (40%); *m/z* (in beam EI) 230 $[M+H]^+$ (5%), 229 $[M]^+$ (4%), 214 $[M-Me]^+$ (10%), 198 $[M+H-CH_2OH]^+$ (100%) X-Ray crystallographic analysis of the hydrochloride of **8** (Fig 1), firmly established both the relative and absolute configuration of **7** The isopropylidene group was readily removed from **8** by aq hydrochloric acid to give the hydrochloride of **7**.

7,7a-Diepialexine (**9**) *or 1,2,3-triepialexine* Oil, $[\alpha]^{20}$ (H_2O, *c* 0 37)· + 11 6° (589), + 13 8° (578), + 15 4° (546), + 21 7° (436), + 25 1° (365) The proton NMR spectrum of 7,7a-diepialexine

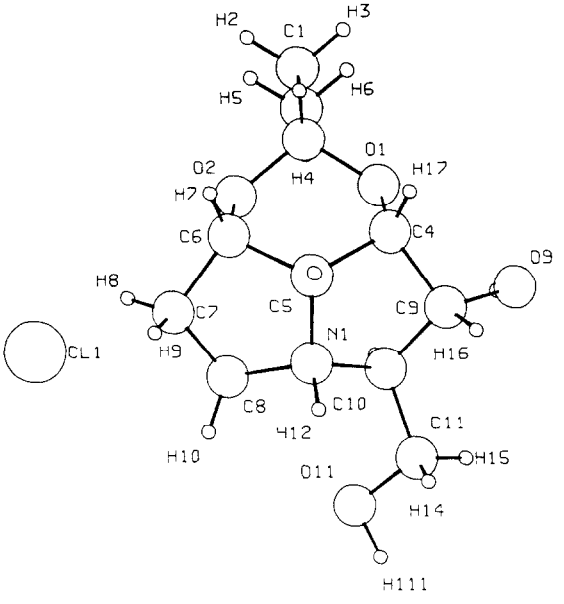

Fig 1 X-Ray molecular structure of hydrochloride of 1,7-*O*-isopropylidene-1,7a-diepialexine (**8**) showing crystallographic numbering scheme, plotted using SNOOPI [7]

Table 3. Bond lengths (Å) for the non-hydrogen atoms in **8** with e.s d 's in parentheses (atomic labelling as in Fig. 1)

C-1	C-3	1 510(4)
C-2	C-3	1 504(4)
C-3	O-1	1 426(3)
C-3	O-2	1 433(3)
C-4	C-5	1 520(4)
C-4	C-9	1 535(3)
C-4	O-1	1 423(3)
C-5	C-6	1 533(3)
C-5	N-1	1 532(3)
C-6	C-7	1 516(4)
C-6	O-2	1 418(4)
C-7	C-8	1 521(4)
C-8	N-1	1 510(3)
C-9	C-10	1 540(3)
C-9	O-9	1 400(3)
C-10	C-11	1 508(3)
C-10	N-1	1 492(4)
C-11	O-11	1 413(3)

Table 4 Bond angles (°) for the non-hydrogen atoms in **8** with e s d 's in parentheses (atomic labelling as in Fig 1)

C-2	C-3	C-1	113 2(2)
O-1	C-3	C-1	112 3(2)
O-1	C-3	C-2	105 4(2)
O-2	C-3	C-1	111 4(2)
O-2	C-3	C-2	105 8(2)
O-2	C-3	O-1	108 3(2)
C-9	C-4	C-5	102 5(2)
O-1	C-4	C-5	113 3(2)
O-1	C-4	C-9	105 8(2)
C-6	C-5	C-4	117 2(2)
N-1	C-5	C-4	106 0(2)
N-1	C-5	C-6	104 4(2)
C-7	C-6	C-5	103 6(2)
O-2	C-6	C-5	110 6(2)
O-2	C-6	C-7	106 9(2)
C-8	C-7	C-6	103 6(2)
N-1	C-8	C-7	103 8(2)
C-10	C-9	C-4	102 9(2)
O-9	C-9	C-4	115 1(2)
O-9	C-9	C-10	114 0(2)
C-11	C-10	C-9	115 3(2)
N-1	C-10	C-9	102 0(2)
N-1	C-10	C-11	112 5(2)
O-11	C-11	C-10	107 7(2)
C-4	O-0	C-3	116 6(2)
C-6	O-2	C-3	114 7(2)
C-8	N-1	C-5	108 0(2)
C-10	N-1	C-5	107 6(2)
C-10	N-1	C-8	117 8(2)

was assigned on the basis of a proton–proton shift correlation (COSY) spectrum and consisted of δ4.19 (*m*, 1H, 7-H), 4.02 (*dd*, 1H, 1-H), 3.70 (*dd*, 1H, 2-H), 3.59 (*dd*, 1H, CHHOH), 3.42 (*dd*, 1H, CHHOH), 2.98 (*dd*, 1H, 7a-H), 2.97 (*m*, 1H, 5-H), 2.50 (*m*, 2H, 3-H and 5'-H), 1.85 (*m*, 1H, 2-H), 1.73 (*m*, 1H, 2'H) ^{13}C NMR α79.7 (*d*), 73.8 (*d*), 71.4 (*d*), 71.2 (*d*), 70.2 (*d*), 63.5 (*t*), 52.5 (*t*), 35.8 (*t*). MS *m/z* (DCI NH_3) 190 $[M+H]^+$ (40%), 158 $[M+H-CH_2OH]^+$ (100%), 112 (35%), 86 (30%), 70 (22%).

7,7a-Diepialexine hydrochloride (**9**). Mp 151–152°. ^{1}H NMR δ4.51 (*dd*, 1H), 4.33 (*dd*, 1H), 3.98 (*dd*, 1H), 3.70 (*m*, 4H), 3.20 (*m*, 2H), 2.10 (*m*, 2H). MS *m/z* (DCI NH_3) 190 $[M+H]^+$ (100%), 158 $[M+H-CH_2OH]^+$ (15%). (Found: C, 42.29; H, 7.12; N, 6.06. $C_8H_{16}NO_4Cl$ requires C, 42.57; H, 7.10; N, 6.21%). Unfortunately, the crystals of 7,7a-diepialexine hydrochloride were not suitable for X-ray crystallographic analysis and the assignment of the relative configurations has been made on the basis of equilibrium NOE experiments on peracetylated **9**. evidence for the stereochemistry of the substituents on the fully substituted five-membered ring was provided by observations of NOE's between substituents in a *cis*-1,3 relationship. Thus NOE's were observed between 7a-H [irradiation of 7a-H generated a 5% enhancement of 2-H, and irradiation of 2-H generated a 2% enhancement of 7a-H], and between 1-H and 3-H [irradiation of 1-H generated a 3% enhancement of 3-H, and irradiation of 3-H generated an 8% enhancement of 1-H], this indicates that all the substituents on the fully substituted five-membered ring are mutually trans to the adjacent substituents. Because the compound is clearly different from australine (**3**), these experiments are consistent with the new alexine being 7,7a-diepialexine (**9**) or its enantiomer (**10**). We are currently attempting an unambiguous synthesis of 7,7a-diepialexine in order to establish the absolute and relative configuration of this material.

Enzyme assays. The reagents and conditions for the assay of enzyme inhibition of mouse gut digestive glucosidase (Table 1) have been reported elsewhere [18]. Glucan 1,4-α-glucosidase (amyloglucosidase) [EC 3.2.1.3] from *Asperaillus niger* [Sigma, Poole] was assayed (Table 2) using amylose from potato [Sigma, Poole], the reaction mixture containing amylose (0.13%), inhibitor and enzyme in 50 mM maleate buffer, pH 6, was incubated for 15 min at 37°. The reaction was stopped by immersion in a boiling water bath for 5 min, followed by incubation for 1 hr at 37° with a tris-glucose oxidase reagent [19] in which the colour reagent was 2,2'-azino-bis(3-ethylbenzthiazoline 6-sulphonic acid). The assay was terminated by addition of 5 M aq. HCl and the absorbance read at 420 nm.

Acknowledgements—We are very grateful to Dr R. J. Molyneux (Agricultural Research Service of U.S. Department of Agriculture) for providing copies of spectra and data for australine prior to publication. Financial support (to RJN) for this work from the Medical Research Council is gratefully acknowledged. Excellent technical assistance was provided by Elaine Porter (Kew) and Peter Witham (Wye).

REFERENCES

1. Nash, R. J., Fellows, L. E., Dring, J. V., Fleet, G. W. J., Derome, A. E., Hamor, T. A., Scofield, A. M. and Watkin, D. J. (1988) *Tetrahedron Letters* **29**, 2487.
2. Nash, R. J., Fellows, L. E., Plant, A. C., Fleet, G. W. J., Derome, A. E., Baird, M. P., Hegarty, M. P. and Scofield, A. M., (1988) *Tetrahedron* **44**, 5959.
3. Molyneux, R. J., Benson, M., Wong, R., Tropea, J. and Elbein, A. D., (1988) *J. Nat. Prod.* **51**, 1198.
4. Nash, R. J., Fellows, L. E., Dring, J. V., Stirton, C. H., Carter, D., Hegarty, M. P. and Bell, E. A. (1988) *Phytochemistry* **27**, 1403.
5. Fellows, L. E., Kite, G. C., Nash, R. J., Simmonds, M. S. J. and Scofield, A. M. (1989) *Plant Nitrogen Metabolism*, (Poulton, J. E., Romeo, J. T. and Conn, E. E., eds), p. 395. Plenum, New York.
6. Fleet, G. W. J., Haraldsson, M., Nash, R. J. and Fellows, L. E. (1988) *Tetrahedron Letters* **29**, 5441.
7. Davies, E. K. (1981) Chemical Crystallography Laboratory, University of Oxford, Baird, P. D. and Foxman, B. (1987) Chemical Crystallography Laboratory, University of Oxford.
8. Fellows, L. E., Kite, G. C., Nash, R. J., Simmonds, M. S. J. and Scofield, A. M. (1989) *Recent Advances in Phytochemistry* (Conn, E. E. ed.) (in press).
9. Fellows, L. E. and Fleet, G. W. J. (1988) *Alkaloidal Glycosidase Inhibitors from Plants in Natural Products Isolation* (Wagman, G. H. and Copper, R., eds), p. 540. Elsevier, Amsterdam.
10. Tropea, J. E., Molyneux, R. J., Kaushal, G. P., Pan, Y. T., Mitchell, M. and Elbein, A. D. (1989) *Biochemistry* **28**, 2027.
11. Nash, R. J., Goldstein, W. S., Evans, S. V. and Fellows, L. E. (1986) *J. Chromatog.* **366**, 431.
12. Sheldrick, G. M. (1985) *Crystallographic Computing* 3 (Sheldrick, G. M., Kruger, C. and Goddard, R. eds) Oxford University Press, Oxford.
13. Watkin, D. J., Carruthers, J. R. and Betteridge, P. W. (1985) *CRYSTALS User Guide* Chemical Crystallography Laboratory, University of Oxford.
14. Anon (1974) *International Tables for X-Ray Crystallography*, Vol. IV. Kynoch Press, Birmingham.
15. Larson, A. C. (1976) Crystallographic Computing Techniques (Ahmed, F. R., ed.) Munksgaard, Copenhagen.
16. Flack, H. D. (1983) *Acta Cryst.* **A39**, 876.
17. Prince, E. (1982) *Mathematical Techniques in Crystallography and Material Sciences* Springer, New York.
18. Scofield, A. M., Fellows, L. E., Nash, R. J. and Fleet, G. W. J. (1986) *Life Sci.* **39**, 645.
19. Dahlqvist, A. (1968) *Anal. Biochem.* **22**, 99.