Acta Crystallographica Section C
Crystal Structure Communications
ISSN 0108-2701

The absolute configuration of 1-epialexine hemihydrate

Amber L. Thompson,[a]* David J. Watkin,[a] Zoltan A. Gal,[b] Laurence Jones,[c] Jackie Hollinshead,[c] Sarah F. Jenkinson,[d] George W. J. Fleet[d] and Robert J. Nash[c]

[a]Department of Chemical Crystallography, Chemical Research Laboratory, Oxford University, Mansfield Road, Oxford OX1 3TA, England, [b]Oxford Diffraction Ltd, 10 Mead Road, Oxford Industrial Park, Yarnton, Oxfordshire OX5 1QU, England, [c]Summit PLC, Plas Gogerddan, Aberystwyth, Ceredigion SY23 3EB, Wales, and [d]Department of Organic Chemistry, Chemical Research Laboratory, Oxford University, Mansfield Road, Oxford OX1 3TA, England
Correspondence e-mail: amber.thompson@chem.ox.ac.uk

Received 3 October 2008
Accepted 10 November 2008
Online 22 November 2008

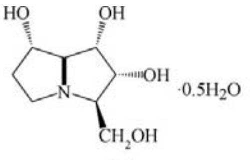
The absolute and relative configurations of 1-epialexine are established by X-ray crystallographic analysis, giving (1*S*,2*R*,3*R*,7*S*,7a*S*)-1,2,7-trihydroxy-3-(hydroxymethyl)pyrrolizidine. The compound crystallizes as the hemihydrate $C_8H_{15}NO_4\cdot 0.5H_2O$, with hydrogen bonds holding the water molecule in a hydrophilic pocket between epialexine bilayers. In addition, a comparison was made between results obtained from examination of the Bijvoet pairs from data sets collected using molybdenum and copper radiation.

Comment

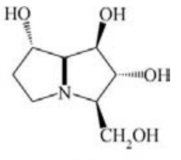
A new polyhydroxylated pyrrolizidine alkaloid, 1-epialexine, (I), has been isolated from the stems of *Castanospermum australe*. Alexine, (II), isolated from *Alexa leiopetala* (Nash *et al.*, 1988), was the first example of a class of 3-hydroxymethyltrihydroxypyrrolizidines, (IV), known as alexines; they possess five chiral centers that give rise to 32 stereoisomers. Australine, (III), which is epimeric at C-7a, was isolated from *Castanospermum australe* (Molyneux *et al.*, 1988) and shown to inhibit various glucosidases (Tropea *et al.*, 1989). Subsequently, other diastereomeric natural products have been isolated (Harris *et al.*, 1989; Nash *et al.*, 1990; Kato *et al.*, 1999, 2007).

The alexines can be regarded as iminosugar analogues which have considerable potential as therapeutic agents (Asano *et al.*, 2000; Watson *et al.*, 2001). 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. Because of the biological activity of the alexines, considerable effort has been expended in synthesizing both natural and unnatural stereoisomers (Choi *et al.*, 1991; Fleet *et al.*, 1988; Takahashi *et al.*, 2008; Trost *et al.*, 2007; Kumar & Pinto, 2006).

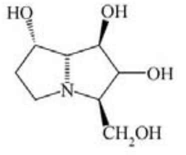
The title compound was found to crystallize in the monoclinic space group *C*2 as the hemihydrate (Fig. 1). The water molecule is well ordered, occupying a position on the twofold axis and acting as a hydrogen-bond acceptor for the hydroxy group O9—H91 and its twofold-related counterpart. The unique water H atom was clearly visible in a difference map hydrogen bonding to atom N4 of a neighbouring 1-epialexine molecule. As a result of these interactions, the O atom of the water molecule occupies a position at the centre of a hydrogen-bonded tetrahedron (Fig. 2). In addition to these interactions, there are a number of other strong intermolecular O—H···O hydrogen bonds (Table 1). These hydrogen bonds lead to the formation of sheets of epialexine molecules perpendicular to the *c* axis. These sheets can be viewed as having a hydrophobic and a hydrophilic side, with all the hydrogen-bonding interactions on the hydrophilic side. The hydrogen bonds connect together pairs of layers with the water molecules in the middle. Thus (I) forms a layered structure of the form *ABA*–*ABA*, where *B* represents the hydrophilic–water component (Fig. 3).



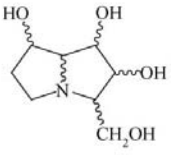
(I)



(II)



(III)



(IV)

The structure was initially determined using Mo *Kα* radiation on a Nonius KappaCCD diffractometer. Friedel pairs were measured and the Flack (1983) *x* parameter refined (Table 2) using the *CRYSTALS* software (Betteridge *et al.*,

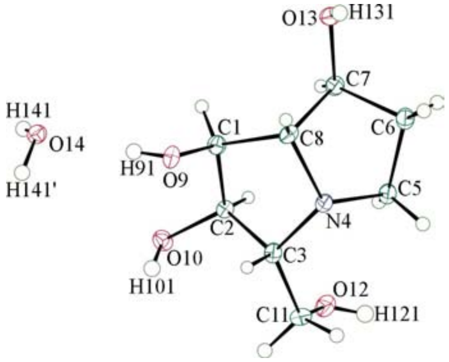


Figure 1
The structure of 1-epialexine hemihydrate from the Gemini–Cu data, shown with displacement ellipsoids drawn at the 50% probability level. [Symmetry code: (′) $-x+1, y, -z+1$.]

doi:10.1107/S0108270108037086

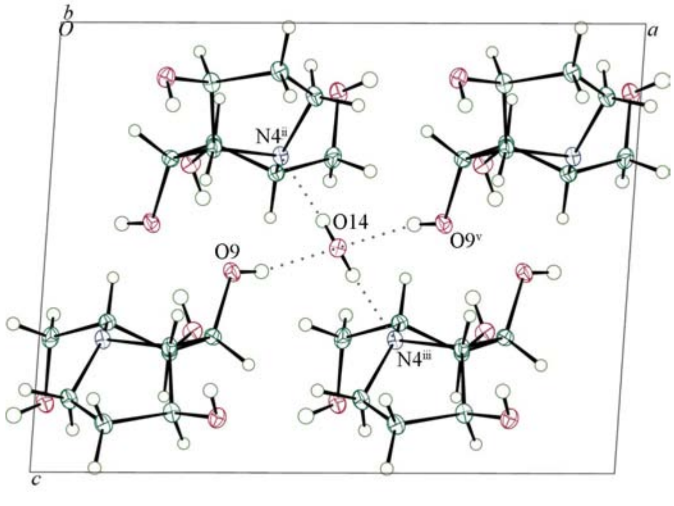


Figure 2
The hydrogen bonding between the 1-epialexine molecule and the water molecule of crystallization in (I), viewed down the unique axis, showing the $C2$ symmetry. The O14$\cdots$N4 distance is 2.737 (2) Å and O14$\cdots$O9 is 2.719 (2) Å for the Gemini–Cu data. [Symmetry codes: (ii) $-x + \frac{3}{2}, y - \frac{1}{2}, -z + 1$; (iii) $x - \frac{1}{2}, y - \frac{1}{2}, z$; (v) $-x + 1, y, -z + 1$.]

2003). The Flack x parameter was outside the conventionally accepted range with a very large s.u., rendering it essentially meaningless. However, examination of the Bijvoet pairs was carried out within *CRYSTALS*, and this gave the Hooft y parameter as −0.7 (4) with G = 2.3 (8) (Hooft *et al.*, 2008). This gave the probability that the absolute configuration was correct as greater than 99% assuming the material to be enantiopure, with the probability of a reliable assignment greater than 90% for a three-hypothesis model (Hooft *et al.*, 2008).

In order to confirm the absolute configuration, the data collection was repeated on the same crystal using an Oxford Diffraction Gemini A Ultra diffractometer and the Enhance Ultra (Cu $K\alpha$) source. For comparison, data were also collected on the same diffractometer using Mo $K\alpha$ radiation and on a second Nonius KappaCCD diffractometer (Mo $K\alpha$). The Flack x parameter, the Hooft y parameter, G (all with s.u. values) and the probabilities derived from these values are given in Table 2.

In all four cases, the s.u. values for the Flack x parameter are greater than the value of 0.10 suggested as the upper limit for confidently determining the absolute configuration of a known enantiopure compound (Flack & Bernardinelli, 2000). However, in order to achieve s.u. values of this magnitude with only carbon, hydrogen, nitrogen and oxygen present in the crystal, the data need to be of exceptional quality. Nonetheless, the likelihood that the absolute structure is incorrect given the data collected with Cu $K\alpha$ radiation seems very small.

Examination of the three cases where data were collected with Mo $K\alpha$ radiation is also interesting. In two of these three examples, the Flack x parameter was considerably outside the meaningful range for the parameter, and for all three, the s.u. value was exceptionally large, indicating that the absolute configuration could not be determined. In contrast, examination of the Bijvoet pairs using the Hooft method suggests that the absolute configuration could be determined with a high confidence.

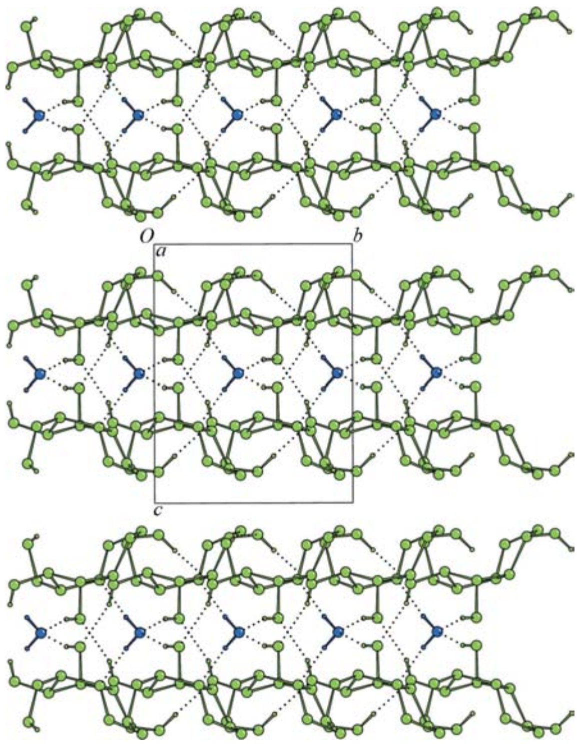


Figure 3
The layered structure of (I) (viewed down the a axis). The hydrogen-bonding interactions are shown as dotted lines.

Experimental

The title compound was isolated from *Castanospermum australe* and crystallized from an ethanol/water/acetone mixture. The melting point was recorded as 432–434 K. $[\alpha]_D^{25}$ +53.4 (c, 0.43 in H_2O).

Compound (I) (KappaCCD 1)

Crystal data

$C_8H_{15}NO_4\cdot 0.5H_2O$
M_r = 198.22
Monoclinic, $C2$
a = 12.4594 (6) Å
b = 7.3115 (4) Å
c = 9.5878 (5) Å
β = 93.843 (2)°
V = 871.45 (8) Å^3
Z = 4
Mo $K\alpha$ radiation
μ = 0.12 mm^{-1}
T = 150 K
0.25 × 0.21 × 0.07 mm

Data collection

Nonius KappaCCD diffractometer
Absorption correction: multi-scan (*DENZO*/*SCALEPACK*; Otwinowski & Minor, 1997)
$T_{\min}$ = 0.91, $T_{\max}$ = 0.99
5649 measured reflections
1943 independent reflections
1863 reflections with $I > 2\sigma(I)$
$R_{\rm int}$ = 0.030

Refinement

$R[F^2 > 2\sigma(F^2)]$ = 0.030
$wR(F^2)$ = 0.071
S = 0.98
1943 reflections
126 parameters
3 restraints
H-atom parameters constrained
$\Delta\rho_{\max}$ = 0.19 e Å^{-3}
$\Delta\rho_{\min}$ = −0.17 e Å^{-3}
Absolute structure: Flack (1983), 875 Friedel pairs
Flack parameter: −1.1 (9)

Compound (**I**) (Gemini–Mo)

Crystal data

$C_8H_{15}NO_4\cdot0.5H_2O$
$M_r = 198.22$
Monoclinic, $C2$
$a = 12.4267\ (3)$ Å
$b = 7.30208\ (18)$ Å
$c = 9.5708\ (2)$ Å
$\beta = 93.839\ (2)^\circ$
$V = 866.51\ (4)$ Å^3
$Z = 4$
Mo $K\alpha$ radiation
$\mu = 0.12$ mm^{-1}
$T = 100$ K
$0.25 \times 0.21 \times 0.07$ mm

Data collection

Oxford Diffraction Gemini A Ultra CCD diffractometer
Absorption correction: multi-scan (*CrysAlis RED*; Oxford Diffraction, 2008)
$T_{\min} = 0.92$, $T_{\max} = 0.99$
7771 measured reflections
1724 independent reflections
1402 reflections with $I > 2\sigma(I)$
$R_{\rm int} = 0.030$

Refinement

$R[F^2 > 2\sigma(F^2)] = 0.026$
$wR(F^2) = 0.064$
$S = 1.01$
1511 reflections
126 parameters
3 restraints
H-atom parameters constrained
$\Delta\rho_{\max} = 0.19$ e Å^{-3}
$\Delta\rho_{\min} = -0.16$ e Å^{-3}
Absolute structure: Flack (1983), 777 Friedel pairs
Flack parameter: −1.5 (9)

Compound (**I**) (Gemini–Cu)

Crystal data

$C_8H_{15}NO_4\cdot0.5H_2O$
$M_r = 198.22$
Monoclinic, $C2$
$a = 12.4190\ (4)$ Å
$b = 7.2933\ (2)$ Å
$c = 9.5671\ (3)$ Å
$\beta = 93.841\ (3)^\circ$
$V = 864.60\ (5)$ Å^3
$Z = 4$
Cu $K\alpha$ radiation
$\mu = 1.05$ mm^{-1}
$T = 100$ K
$0.25 \times 0.21 \times 0.07$ mm

Data collection

Oxford Diffraction Gemini A Ultra CCD diffractometer
Absorption correction: multi-scan (*CrysAlis RED*; Oxford Diffraction, 2008)
$T_{\min} = 0.80$, $T_{\max} = 0.92$
7817 measured reflections
1479 independent reflections
1464 reflections with $I > 2\sigma(I)$
$R_{\rm int} = 0.025$

Refinement

$R[F^2 > 2\sigma(F^2)] = 0.026$
$wR(F^2) = 0.071$
$S = 1.06$
1476 reflections
126 parameters
3 restraints
H-atom parameters constrained
$\Delta\rho_{\max} = 0.19$ e Å^{-3}
$\Delta\rho_{\min} = -0.15$ e Å^{-3}
Absolute structure: Flack (1983), 650 Friedel pairs
Flack parameter: −0.01 (17)

Table 1
Hydrogen-bond geometry (Å, °) for (I) (Gemini–Cu).

D—H···A	D—H	H···A	D···A	D—H···A
C5—H52···O12	0.97	2.40	3.009 (2)	121
C6—H61···O12$^{\rm i}$	0.97	2.54	3.379 (2)	145
O13—H131···O10$^{\rm i}$	0.84	1.90	2.739 (2)	177
O10—H101···O9$^{\rm ii}$	0.79	2.00	2.758 (2)	159
O14—H141···N4$^{\rm iii}$	0.83	1.91	2.737 (2)	172
O9—H91···O14	0.80	1.94	2.719 (2)	163
O12—H121···O13$^{\rm iv}$	0.79	2.07	2.825 (2)	160

Symmetry codes: (i) $x, y+1, z$; (ii) $-x+\frac{3}{2}, y-\frac{1}{2}, -z+1$; (iii) $x-\frac{1}{2}, y-\frac{1}{2}, z$; (iv) $x+\frac{1}{2}, y-\frac{1}{2}, z$.

Table 2
The values for the Flack *x* parameter, the Hooft *y* parameter, *G* (with s.u.) and the probabilities[a] derived from these values.

	KappaCCD 1	Gemini–Mo	Gemini–Cu	KappaCCD 2
Flack *x*	−1.1 (9)	−1.5 (9)	−0.01 (17)	−0.1 (8)
Hooft *y*	−0.7 (4)	−1.7 (3)	−0.03 (2)	−0.1 (4)
G	2.3 (8)	4.4 (5)	1.06 (4)	1.2 (8)
*P*2(correct)[a]	0.999	n/a	1.000	0.974
*P*3(correct)[a]	0.933	0.992	1.000	0.726
*P*3(*rac*-twin)[a]	0.066	0.008	10^{-7}	0.255
*P*3(inverse)[a]	0.001	0.2×10^{-4}	10^{-7}	0.019
Reflections	1943	1724	1476	1974
Friedel pairs	875	777	650	902

Note: (*a*) *P*2(correct) is the probability that the given enantiomer is correct assuming that the crystal is enantiopure. The *P*3 probabilities assume a three possibility hypothesis, which adds the third possibility that the crystal is a racemic twin.

Compound (**I**) (KappaCCD 2)

Crystal data

$C_8H_{15}NO_4\cdot0.5H_2O$
$M_r = 198.22$
Monoclinic, $C2$
$a = 12.4567\ (5)$ Å
$b = 7.3097\ (3)$ Å
$c = 9.5920\ (5)$ Å
$\beta = 93.8151\ (16)^\circ$
$V = 871.46\ (7)$ Å^3
$Z = 4$
Mo $K\alpha$ radiation
$\mu = 0.12$ mm^{-1}
$T = 150$ K
$0.25 \times 0.21 \times 0.07$ mm

Data collection

Nonius KappaCCD diffractometer
Absorption correction: multi-scan (*DENZO*/*SCALEPACK*; Otwinowski & Minor, 1997)
$T_{\min} = 0.94$, $T_{\max} = 0.99$
7632 measured reflections
1974 independent reflections
1862 reflections with $I > 2\sigma(I)$
$R_{\rm int} = 0.032$

Refinement

$R[F^2 > 2\sigma(F^2)] = 0.029$
$wR(F^2) = 0.067$
$S = 1.01$
1974 reflections
126 parameters
3 restraints
H-atom parameters constrained
$\Delta\rho_{\max} = 0.34$ e Å^{-3}
$\Delta\rho_{\min} = -0.33$ e Å^{-3}
Absolute structure: Flack (1983), 902 Friedel pairs
Flack parameter: −0.1 (8)

A colourless single crystal was mounted in a nylon loop using perfluoropolyether oil and quench-cooled to 150 K in a stream of cold N_2 using an Oxford Cryosystems Cryostream unit (Cosier & Glazer, 1986). Diffraction data were initially measured using a Nonius KappaCCD diffractometer (graphite-monochromated Mo $K\alpha$ radiation). Given that the compound was known to be enantiopure, examination of the systematic absences of the intensity data showed the space group to be $C2$.

The H atoms were all visible in a difference map and were refined with soft restraints on the bond lengths and angles to regularize their geometry (C—H in the range 0.93–0.98 Å), and with $U_{\rm iso}$(H) in the range 1.2–1.5 times $U_{\rm eq}$ of the parent atom, after which the positions were refined with riding constraints.

The Flack *x* parameter was refined and the Bijvoet pairs examined to give the Hooft *y* parameter, *G* and the probabilities that the absolute configuration was correct as explained above (Hooft *et al.*, 2008; Table 2).

The crystal was then remounted on an Oxford Diffraction Gemini A Ultra at 100 K, where data were collected using both Mo $K\alpha$ and Cu $K\alpha$ radiation. The atomic coordinates from the initial structure determination were refined against the data as above. Data were similarly collected on a second Nonius KappaCCD diffractometer (graphite-monochromated Mo $K\alpha$ radiation) and the model refined.

For the KappaCCD data collections, data collection: *COLLECT* (Nonius, 2001); cell refinement: *DENZO/SCALEPACK* (Otwinowski & Minor, 1997); data reduction: *DENZO/SCALEPACK*. For the Gemini data collections, data collection: *CrysAlis CCD* (Oxford Diffraction, 2008); cell refinement: *CrysAlis RED* (Oxford Diffraction, 2008); data reduction: *CrysAlis RED*. For all data collections, program(s) used to solve structure: *SIR92* (Altomare *et al.*, 1994); program(s) used to refine structure: *CRYSTALS* (Betteridge *et al.*, 2003); molecular graphics: *CAMERON* (Watkin *et al.*, 1996); software used to prepare material for publication: *CRYSTALS*.

Richard I. Cooper (oXray Ltd) and other colleagues are thanked for helpful discussions.

Supplementary data for this paper are available from the IUCr electronic archives (Reference: LN3116). Services for accessing these data are described at the back of the journal.

References

Altomare, A., Cascarano, G., Giacovazzo, C., Guagliardi, A., Burla, M. C., Polidori, G. & Camalli, M. (1994). *J. Appl. Cryst.* **27**, 435.

Asano, N., Nash, R. J., Molyneux, R. J. & Fleet, G. W. J. (2000). *Tetrahedron Asymmetry*, **11**, 1645–1680.

Betteridge, P. W., Carruthers, J. R., Cooper, R. I., Prout, K. & Watkin, D. J. (2003). *J. Appl. Cryst.* **36**, 1487.

Choi, S., Bruce, I., Fairbanks, A. J., Fleet, G. W. J., Jones, A. H., Nash, R. J. & Fellows, L. E. (1991). *Tetrahedron Lett.* **32**, 5517–5520.

Cosier, J. & Glazer, A. M. (1986). *J. Appl. Cryst.* **19**, 105–107.

Flack, H. D. (1983). *Acta Cryst.* A**39**, 876–881.

Flack, H. D. & Bernardinelli, G. (2000). *J. Appl. Cryst.* **33**, 1143–1148.

Fleet, G. W. J., Haraldsson, M., Nash, R. J. & Fellows, L. E. (1988). *Tetrahedron Lett.* **29**, 5441–5445.

Harris, C. M., Harris, T. M., Molyneux, R. J., Tropea, J. E. & Elbein, A. D. (1989). *Tetrahedron Lett.* **30**, 5685–5688.

Hooft, R. W. W., Straver, L. H. & Spek, A. L. (2008). *J. Appl. Cryst.* **41**, 96–103.

Kato, A., Adachi, I., Miyauchi, M., Ikeda, K., Komae, T., Kizu, H., Kameda, Y., Watson, A. A., Nash, R. J., Wormald, M. R., Fleet, G. W. J. & Asano, N. (1999). *Carbohydr. Res.* **316**, 95–103.

Kato, A., Kano, E., Adachi, I., Molyneux, R. J., Watson, A. A., Nash, R. J., Fleet, G. W. J., Wormald, M. R., Kizu, H., Ikeda, K. & Asano, N. (2003). *Tetrahedron Asymmetry*, **14**, 325–331.

Kato, A., Kato, N., Adachi, I., Hollinshead, J., Fleet, G. W. J., Kuriyama, C., Ikeda, K., Asano, N. & Nash, R. J. (2007). *J. Nat. Prod.* **70**, 993–997.

Kumar, N. S. & Pinto, B. M. (2006). *J. Org. Chem.* **71**, 2935–2943.

Molyneux, R. J., Benson, M., Wong, R. Y., Tropea, J. E. & Elbein, A. D. (1988). *J. Nat. Prod.* **51**, 1198–1206.

Nash, R. J., Fellows, L. E., Dring, J. V., Fleet, G. W. J., Derome, A. E., Hamor, T. A., Scofield, A. M. & Watkin, D. J. (1988). *Tetrahedron Lett.* **29**, 2487–2490.

Nash, R. J., Fellows, L. E., Girdhar, A., Fleet, G. W. J., Peach, J. M., Ramsden, N. G., Hegarty, M. P. & Scofield, A. M. (1990). *Phytochemistry*, **29**, 111–114.

Nonius (2001). *COLLECT*. Nonius BV, Delft, The Netherlands.

Otwinowski, Z. & Minor, W. (1997). *Methods in Enzymology*, Vol. 276, *Macromolecular Crystallography*, Part A, edited by C. W. Carter Jr & R. M. Sweet, pp. 307–326. New York: Academic Press.

Oxford Diffraction (2008). *CrysAlis CCD* and *CrysAlis RED*. Versions 1.171.32.24. Oxford Diffraction Ltd, Yarnton, Oxfordshire, England.

Takahashi, M., Maehara, T., Sengoku, T., Fujita, N., Takabe, K. & Yoda, H. (2008). *Tetrahedron*, **64**, 5254–5261.

Tropea, J. E., Molyneux, R. J., Kaushal, G. P., Pan, Y. T., Mitchell, M. & Elbein, A. D. (1989). *Biochemistry*, **28**, 2027—2034.

Trost, B. M., Aponick, A. & Stanzl, B. N. (2007). *Chem. Eur. J.* **13**, 9547–9560.

Watkin, D. J., Prout, C. K. & Pearce, L. J. (1996). *CAMERON*. Chemical Crystallography Laboratory, Oxford, England.

Watson, A. A., Fleet, G. W. J., Asano, N., Molyneux, R. J. & Nash, R. J. (2001). *Phytochemistry*, **56**, 265–295.

Wormald, M. R., Nash, R. J., Hornicar, P., White, J. D., Molyneux, R. J. & Fleet, G. W. J. (1998). *Tetrahedron Asymmetry*, **9**, 2549–2558.