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Redetermination of skimmianine: a new inhibitor against the Leishmania APRT enzyme

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Key indicators

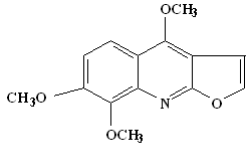
Single-crystal X-ray study
T = 120 K
Mean σ(C–C) = 0.002 Å
R factor = 0.038
wR factor = 0.104
Data-to-parameter ratio = 12.9

For details of how these key indicators were automatically derived from the article, see http://journals.iucr.org/e.

The title compound (alternative names 7,8-dimethoxydictammine and 4,7,8-trimethoxyfuro[2,3-*b*]quinoline), $C_{14}H_{13}NO_4$, is a natural product extracted from *Adiscanthus fusciflorus* (*Rutaceae*). Our biochemical tests show that it has inhibitory activity against the enzyme adenine phosphoribosyltransferase (APRT) from Leishmania, a tropical parasite causing endemic disease in poor countries. It crystallizes in the centrosymmetric space group $P2_1/c$, with one molecule in the asymmetric unit, and has at least two C—H···O intermolecular interactions, leading to the formation of centrosymmetric dimers.

Comment

Leishmaniasis is a disease caused by a protozoal parasite of the order Kinetoplastid. According to the World Heath Organization reports (WHO, 1998), 88 countries are affected, with 12 million infected people and approximately 350 million people at risk. The need for new drugs for the treatment of the leishmaniasis infections comes from a lack of safe drugs and the serious secondary effects observed in available chemotherapy (McGreevy & Marsden, 1986). The purine nucleotide salvage pathway in Kinetoplastid is a potential target for the development of new drugs, owing to its dependence on that biosynthetic pathway (Berens *et al.*, 1995). In Kinetoplastid, the phosphoribosyltransferase (PRTase) protein family is responsible for purine nucleotide salvage. Looking for new bioactive substances, potentially useful against leishmaniasis, we used the PRTase adenine phosphoribosyltransferase (APRT) from *L. tarentolae* as a model system to screen the inhibitory capacity of several small molecule compounds from Brazilian plants.



(I)

The screening was performed using the APRT inhibitory assay, either in the presence of extracts or with the purified compound, and was monitored spectrophotometrically (Tuttle & Krenitsky, 1980). The title compound, (I), was isolated from *A. fusciflorus* extracts and has been structurally investigated because of its inhibitory activity against APRT. Enzymatic tests of (I) at 50 µg/ml show an inhibition activity of 68%. Further investigations by molecular docking and dynamic simulations will be performed to study the interactions

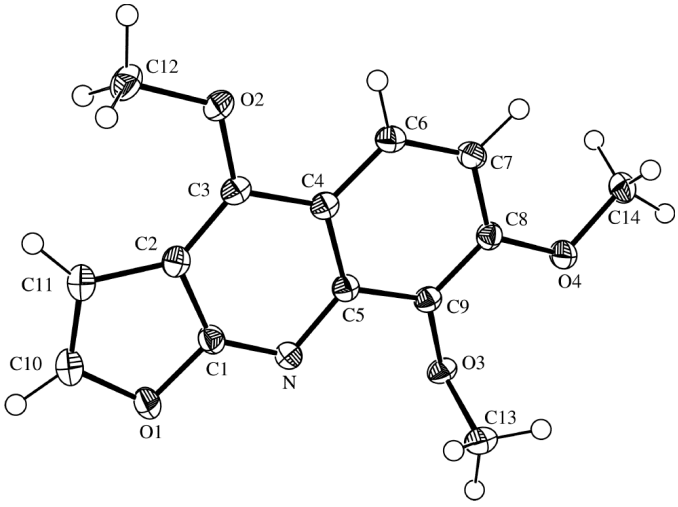


Figure 1
A view of the molecular structure of (I), showing the atom-labelling scheme. Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as spheres of arbitrary radii.

between this compound and the APRT active site. In light of this interest, structural characterization will give us important information with respect to the interaction mode of compound (I) with APRT, and allow the investigation of possible inhibition of that compound by other PRTases.

With the aim of obtaining more accurate structural data for comparison of (I) and other inhibitors against APRT enzyme, and in order to use this information in molecular docking and dynamic simulations, we undertook a structure determination at 120 K. An *ORTEP* view (Farrugia, 1997) of compound (I), together with the atom-labelling scheme, is shown in Fig. 1. All bond lengths and angles of this compound are close to normal values (Allen *et al.*, 1983). The crystallographic structure of (I), measured at room temperature, has been previously published (Cox *et al.*, 1989).

The crystal packing of (I) does not show any strong hydrogen bonds. Nevertheless, two weak intermolecular interactions of the type C—H···O (C10—H10···O3^{i}, C12—H12···O3^{ii}) and two of the type C—H···N (C10—H10···N^{i} and C14—H14···N^{iii}) stabilize the three-dimensional structure (symmetry codes as in Table 1). The latter type links two neighbouring molecules in a centrosymmetric dimeric form, as shown in Fig. 2. The C10—H10···O3^{i} interaction is responsible for the formation of infinite chains along the *b* axis, and C12—H12···O3^{ii} for the formation of infinite chains along the *c* axis. All structural details of the intermolecular contacts for compound (I) were interpreted as hydrogen bonds on geometrical grounds (Ellena *et al.*, 2001).

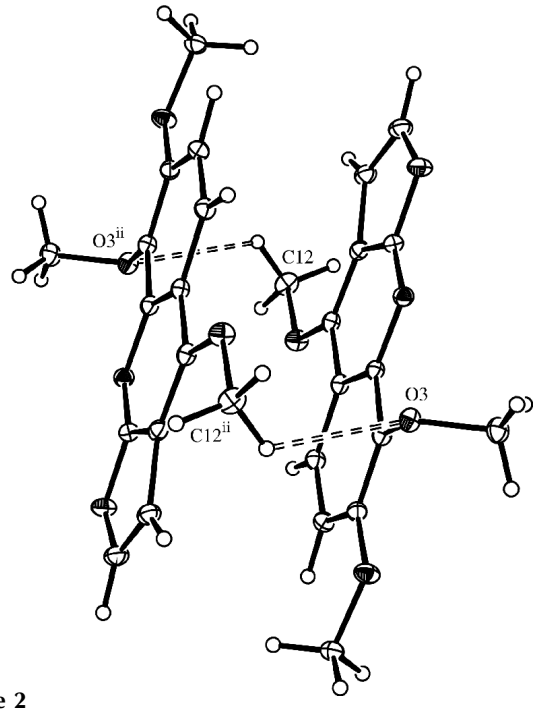


Figure 2
A view of (I), showing dimerization due to C12—H12···O3^{ii} [symmetry code: (ii) $1-x, 1-y, -z$] interactions.

Experimental

The roots and leaves of *A. fusciflorus* were collected from the Manaus region of the Brazilian Amazon forest in December 2000. An authenticated specimen was deposited in the herbarium of the Instituto de Pesquisas da Amazonia–INPA (code 189859). The powdered parts (roots 2.380 kg and leaves 1.040 kg) were extracted successively with hexane (10 l) and methanol (8.5 l). The crude hexane crude extract of the root (5.0 g) was extracted by chromatography on a silica gel column ($\Phi \times h = 28 \times 2$ cm). Fractions 6 and 7 were combined and subjected to silica gel column chromatography, using the isocratic system (hexane/ethyl acetate 7:3). Fraction 8 was collected according to TLC analysis (normal phase) and fractions 6 and 7 were combined and subjected to CCDP using hexane/ethyl acetate 7:3 as the mobile phase. 10 mg of compound (I) were obtained; this crystallized by vapour diffusion as a prismatic, light yellow solid, using 1:1 hexane/dichloromethane as solvent.

Crystal data

$C_{14}H_{13}NO_4$	$D_x = 1.477$ Mg m^{-3}
$M_r = 259.25$	Mo $K\alpha$ radiation
Monoclinic, $P2_1/c$	Cell parameters from 2808 reflections
$a = 7.2429$ (1) Å	$\theta = 3.4$–$27.5°$
$b = 10.4418$ (2) Å	$\mu = 0.11$ mm^{-1}
$c = 15.4618$ (3) Å	$T = 120$ (2) K
$\beta = 94.353$ (1)°	Prism, light yellow
$V = 1165.99$ (4) Å^3	0.32 × 0.18 × 0.16 mm
$Z = 4$	

Data collection

Nonius KappaCCD diffractometer	$R_{int} = 0.013$
φ and ω scans	$\theta_{max} = 27.5°$
Absorption correction: none	$h = -9 \rightarrow 9$
5117 measured reflections	$k = -13 \rightarrow 13$
2672 independent reflections	$l = -20 \rightarrow 20$
2301 reflections with $I > 2\sigma(I)$	

Refinement

Refinement on F^2	$w = 1/[\sigma^2(F_o^2) + (0.0633P)^2 + 0.2061P]$ where $P = (F_o^2 + 2F_c^2)/3$
$R[F^2 > 2\sigma(F^2)] = 0.038$	$(\Delta/\sigma)_{max} < 0.001$
$wR(F^2) = 0.104$	$\Delta\rho_{max} = 0.29$ e Å^{-3}
$S = 1.04$	$\Delta\rho_{min} = -0.22$ e Å^{-3}
2672 reflections	
207 parameters	
H atoms treated by a mixture of independent and constrained refinement	

Table 1
Hydrogen-bonding geometry (Å, °).

D—H···A	D—H	H···A	D···A	D—H···A
C10—H10···O3^{i}	0.97 (1)	2.47 (1)	3.315 (1)	144.8 (11)
C12—H12A···O3^{ii}	0.99 (1)	2.54 (1)	3.159 (1)	120.3 (11)
C10—H10···N^{i}	0.97 (1)	2.53 (1)	3.290 (1)	134.9 (11)
C14—H14A···N^{iii}	0.99 (1)	2.69 (1)	3.403 (1)	128.0 (11)

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

All of the H atoms, except those attached to the C atom C13, were found in a Fourier synthesis and subsequently refined freely. The H atoms attached to C13 were placed at calculated positions.

Data collection: *COLLECT* (Nonius, 1998); cell refinement: *HKL SCALEPACK* (Otwinowski & Minor, 1997); data reduction: *HKL DENZO* (Otwinowski & Minor, 1997) and *SCALEPACK*; program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1997); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *ORTEP*-3 *for Windows* (Farrugia, 1997) and *PLATON* (Spek, 2002); software used to prepare material for publication: *WinGX* publication routines (Farrugia, 1999).

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References

Allen, F. H., Kennard, O. & Taylor, R. (1983). *Acc. Chem. Res.* **16**, 146–153.
Berens, R., Krug, R. & Marr, J. J. (1995). *Biochemistry and Molecular Biology of Parasites*, edited by J. J. Marr and M. Müller, p. 89–118. London: Academic Press Ltd.
Cox, O., Steiner, J. R., Barnes, C. L. & retamozo, H. R. (1989). *Acta Cryst.* C**45**, 1263–1265.
Ellena, J., Goeta, A. E., Howard, J. A. K. & Punte, G. (2001). *J. Phys. Chem.* *A***105**, 8696–8708.
Farrugia, L. J. (1997). *J. Appl. Cryst.* **30**, 565.
Farrugia, L. J. (1999). *J. Appl. Cryst.* **32**, 837–838.
McGreevy, P. B. & Marsden, P. D. (1986). *Chemotheraphy of Parasitic Diseases*, edited by W. C. Campbell and R. S. Rew, Vol. 1, p. 115–127. New York: Plenum Press.
Nonius (1998). *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 and R. M. Sweet, pp. 307–326. New York: Academic Press.
Sheldrick, G. M. (1997). *SHELXS*97 and *SHELXL*97. University of Göttingen, Germany.
Spek, A. L. (2002) *PLATON*. Utrecht University, Utrecht, The Netherlands.
Tuttle, J. V. & Krenitsky, T. A. (1980). *J. Biol. Chem.* 255(3), 909–916.
WHO (1998). World Heath Organization. http://www.who.Int/tdr/diseases/leish/diseaseinfo.htm