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Redetermination of rifampicin pentahydrate revealing a zwitterionic form of the antibiotic

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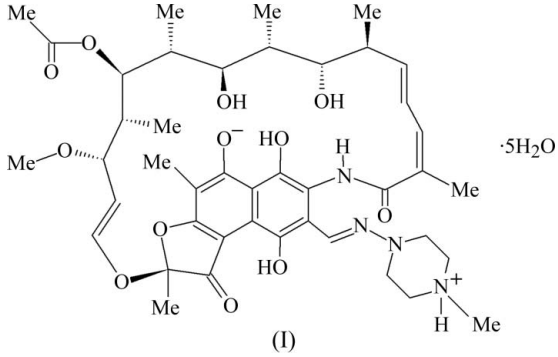
Rifampicin belongs to the family of naphthalenic ansamycin antibiotics. The first crystal structure of rifampicin in the form of the pentahydrate was reported in 1975 [Gadret, Goursolle, Leger & Colleter (1975). *Acta Cryst.* B**31**, 1454–1462] with the rifampicin molecule assumed to be neutral. Redetermination of this crystal structure now shows that one of the phenol –OH groups is deprotonated, with the proton transferred to a piperazine N atom, confirming earlier spectroscopic results that indicated a zwitterionic form for the molecule, namely (2*S*,12*Z*,14*E*,16*S*,17*S*,18*R*,19*R*,20*R*,21*S*,22*R*,23*S*,24*E*)-21-acetyloxy-6,9,17,19-tetrahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-[(*E*)-*N*-(4-methylpiperazin-4-ium-1-yl)formimidoyl]-1,11-dioxo-1,2-dihydro-2,7-(epoxypentadeca[1,11,13]trienimino)naphtho[2,1-*b*]furan-5-olate pentahydrate, $C_{43}H_{58}N_4O_{12}\cdot 5H_2O$. The molecular structure of this antibiotic is stabilized by a system of four intramolecular O—H···O and N—H···N hydrogen bonds. Four of the symmetry-independent water molecules are arranged *via* hydrogen bonds into helical chains extending along [100], whereas the fifth water molecule forms only one hydrogen bond, to the amide group O atom. The rifampicin molecules interact *via* O—H···O hydrogen bonds, generating chains along [001]. Rifampicin pentahydrate is isostructural with recently reported rifampicin trihydrate methanol disolvate.

Comment

Rifampicin, an antibiotic from the group of rifamycins, is utilized worldwide as a result of its broad spectrum of antibacterial activity. Owing to its pronounced activity against mycobacteria, this drug is the first-line antibiotic in the treatment of tuberculosis (Floss & Yu, 2005, and references therein). The solid-state physicochemical properties of rifampicin have been the subject of numerous studies. Two polymorphic forms and a variety of solvates have been reported and characterized for this antibiotic (Ferrari & Gallo, 1975; Pelizza *et al.*, 1977; Henwood *et al.*, 2001; Agrawal *et al.*, 2004). The first structural model of the rifampicin molecule was provided by the crystal structure of rifampicin pentahydrate (Gadret *et al.*, 1975). Although the importance of this model for structural studies is hard to overestimate, as it has been employed in modelling the rifampicin molecule in complexes with proteins (Campbell *et al.*, 2001; Chrencik *et al.*, 2005; Baysarowich *et al.*, 2008), severe problems in locating the H atoms obviated any consideration of a zwitterionic form and the molecule was assumed to be neutral. The possibility of intramolecular proton transfer from the phenolic group at C8 to the piperazine N—CH_3 unit was considered for the first time by Ferrari & Gallo (1975). Later on, based on IR spectroscopic studies, the zwitterionic form was postulated for some rifampicin solvates, including the pentahydrate form (Pelizza *et al.*, 1977). Nevertheless, intramolecular proton transfer in rifampicin is largely neglected in the literature. In a recent report of two rifampicin solvates with ethylene glycol, due to the rather poor quality of the diffraction data, no conclusions were drawn with respect to the zwitterionic *versus* neutral form of rifampicin in these systems (de Villiers *et al.*, 2011). In our recent paper on rifampicin and its analogues, where both the neutral and zwitterionic forms of rifampicin were reported in its solvates, intramolecular proton transfer from the phenolic group to the piperazine N—CH_3 unit was judged to be an important factor in the mechanism of inhibition of DNA-dependent RNA polymerase (RNAP) by this antibiotic (Pyta *et al.*, 2012).



(I)

As a contribution to the further understanding of this important drug, we have redetermined the crystal structure of rifampicin pentahydrate, (I). The rifampicin molecule (Fig. 1) in the pentahydrate form exists as a zwitterion, with the phenolate group at C8 and the proton attached to the piperazine N—CH_3 unit. This proton transfer is not only confirmed by the location of the H atom on piperazine atom N40 through the diffraction data, but also corroborated by the molecular geometry, mainly the C8—O8 bond length of 1.283 (4) Å and piperazine N40—C bond lengths in the range 1.490 (4)–1.498 (4) Å. These geometric parameters are, within experimental error, the same as those reported for the zwitterionic form of rifampicin in its trihydrate methanol disolvate [1.287 (4) and 1.490 (4)–1.493 (4) Å, respectively; Pyta *et al.*, 2012]. The molecular structure of this antibiotic is stabilized by a system of intramolecular hydrogen bonds between: (i) the

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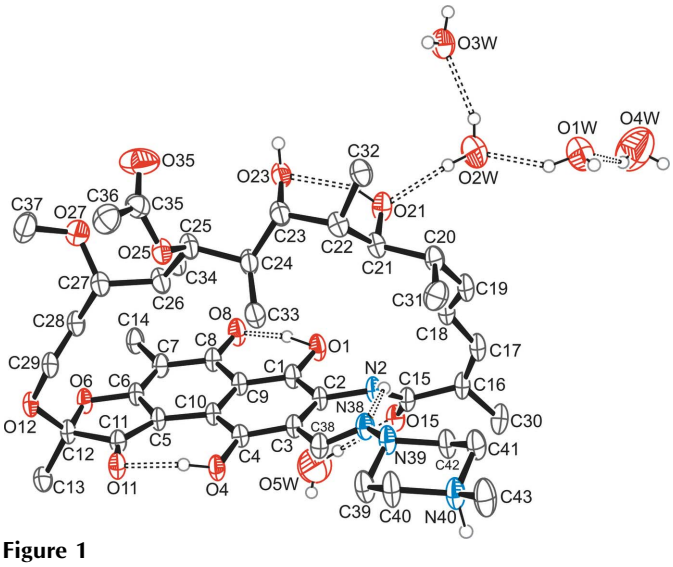

Figure 1
The asymmetric unit of (I), showing the atom-numbering scheme. Displacement ellipsoids are drawn at the 50% probability level. C-bound H atoms have been omitted. Hydrogen bonds are represented by dashed lines.

hydroxy group at C1 and the phenolate group at C8; (ii) the hydroxy group at C4 and the O atom of the carbonyl group at C11; (iii) the amide N—H group and the hydrazide N38 atom; and (iv) in the *ansa* chain, the two hydroxy groups at C21 and C23 (Table 1 and Fig. 1). In the zwitterionic form of rifampicin, a short intramolecular hydrogen bond between the hydroxy group at C1 and the amide carbonyl group (O15) is broken, interrupting the collective system of three hydrogen bonds (O—H···O–H···O=C—N—H···N) observed in the neutral form, and thus giving rise to an increased conformational flexibility about the bond (N2—C2) from the amide group to the aromatic ring.

It has been postulated that, in order to be active against bacterial pathogens, rifamycins need a naphthalene ring with quinone or hydroquinone O atoms at C1 and C8 (using the atom numbering from the present analysis) and two hydroxy groups at C21 and C23 on the *ansa* fragment that have to be in a particular spatial arrangement (Bacchi *et al.*, 1998). In active forms, the O···O distances between the pairs of atoms O1/O8 and O21/O23 should be in the range 5.41–9.58 Å. In inactive forms, the dihedral angle between the best plane through the chromophore and the *ansa* chain should be less than 50° (Bacchi *et al.*, 1998). In rifampicin pentahydrate, (I), the O1···O21, O1···O23, O8···O21 and O8···O23 distances are 5.346 (4), 6.123 (4), 6.870 (4) and 6.759 (4) Å, respectively, and the relevant dihedral angle is 86.7 (1)°. The etheric junction, spanning atoms C12, O12, C29 and C28, is almost perpendicular to the chromophore, with the dihedral angle between their best planes being 86.5 (1)°. In turn, the absence of an intramolecular hydrogen bond between amide atom O15 and the hydroxy group at C1 results in strong twisting of the amide group relative to the chromophore, with a dihedral angle of 55.6 (2)° between their best planes. This rotation, placing the amide carbonyl group on the other side of the naphthalene ring from that occupied by the *ansa* chain, results in a few

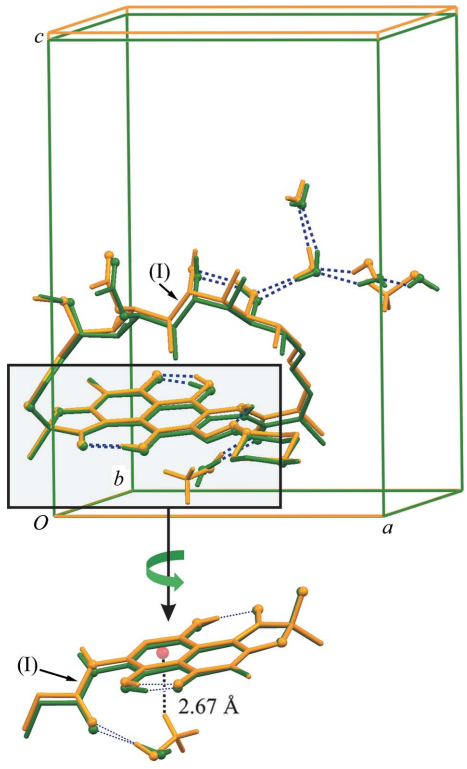

Figure 2
Superposition of the crystal structures of rifampicin pentahydrate (green in the electronic version of the paper), (I), and rifampicin trihydrate methanol disolvate (orange) with a common origin of the unit cells. A view of the superposition of the naphthalene fragments is shown separately at the bottom.

short intermolecular contacts for amide atom O15, including an O—H···O hydrogen bond with water molecule O5*W* and two short C—H···O contacts with methyl groups (Table 1). The piperazine ring, as expected, adopts a chair conformation, with the two substituents at the piperazine N atoms situated equatorially. The overall conformational parameters of the rifampicin molecule in its pentahydrate form in (I) are in good agreement with those found for rifampicin trihydrate methanol disolvate (Pyta *et al.*, 2012) (Fig. 2). The r.m.s. deviation of the least-squares superposition of rifampicin molecules from these two solvates is 0.133 Å, as calculated using the program *COOT* (Emsley *et al.*, 2010).

For compound (I) as a whole, rifampicin pentahydrate is isostructural with rifampicin trihydrate methanol disolvate (Pyta *et al.*, 2012), where water molecules O4*W* and O5*W* of the present structure are replaced by two methanol molecules without significantly altering the crystal packing (Fig. 3). In both solvates, the rifampicin molecules are connected directly by a hydrogen bond between the O23—H23 hydroxy group of the *ansa* chain and the O atom of the O4—H4 hydroxy group of the naphthalene ring in the molecule at $(-x+\frac{1}{2}, -y+1, z+\frac{1}{2})$, forming a chain extending along [001]. Also, in the 1,1,1-trichloroethane solvate (Pyta *et al.*, 2012), the neutral rifampicin molecules are assembled into chains *via* interaction of the same donor group with the more basic piperazine N40 atom as an acceptor. In the pentahydrate form, (I), four water

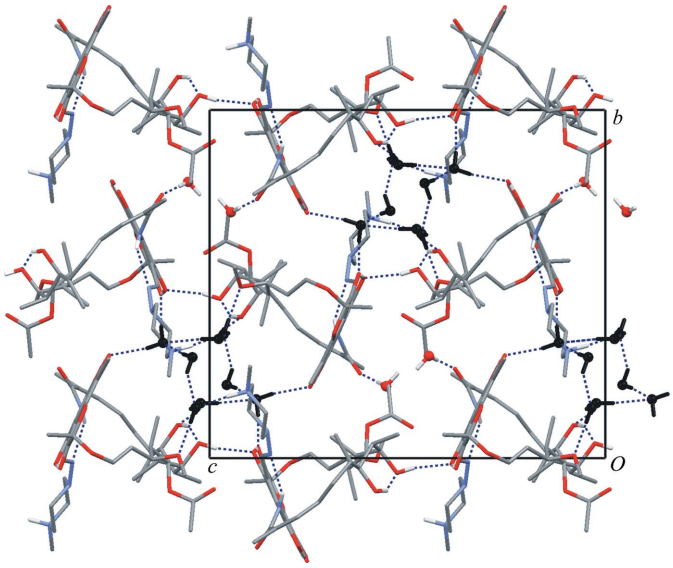

Figure 3
The packing of (I), viewed along [100]. The water molecules forming helical chains along [100] are shown in black. C-bound H atoms are not shown. Hydrogen bonds are represented by dashed lines.

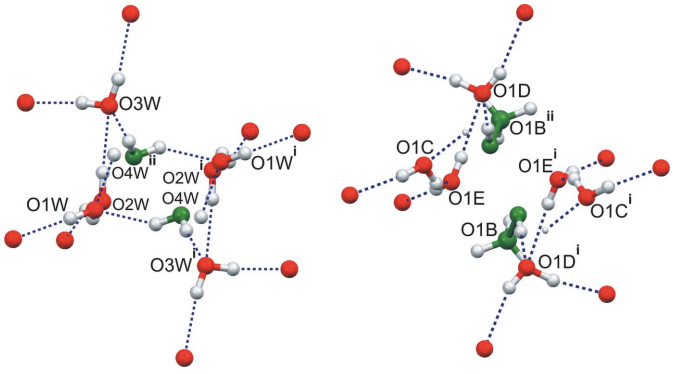

Figure 4
A comparison of the helical water chain in rifampicin pentahydrate, (I) (left), and a similar fragment in rifampicin trihydrate methanol disolvate (right), with water molecule O4*W* (green in the electronic version of the journal) replaced by a methanol molecule (green). Hydrogen bonds are represented by dashed lines. [Symmetry codes: (i) $x+\frac{1}{2}, -y+\frac{3}{2}, -z+1$; (ii) $x-\frac{1}{2}, -y+\frac{3}{2}, -z+1$.]

molecules, O1*W* to O4*W*, are hydrogen bonded into a helical chain extending along [100], with water molecules O1*W* and O2*W* each acting as a single donor and a single acceptor, O3*W* as a double acceptor and O4*W* as a double donor of hydrogen bonds within this chain (Figs. 3 and 4). Water molecules O1*W*, O2*W* and O3*W* are additionally hydrogen bonded to rifampicin *via* five hydrogen bonds, one of them involving the piperazinium N40—H40 group as donor and atom O1*W*($-x+\frac{3}{2}, -y+1, z-\frac{1}{2}$) as acceptor. In turn, water molecule O5*W* is located in a hydrophobic niche over the naphthalene group lined by methyl groups of rifampicin molecules belonging to two neighbouring [001] chains. Atom O5*W* is only weakly hydrogen bonded, forming one hydrogen bond to the amide carbonyl group. When this water molecule is replaced by methanol in the trihydrate dimethanol solvate, the position of the O atom remains virtually unchanged and the methyl group is directed to the naphthalene ring system with a methyl H atom at a distance of 2.67 Å from the naphthalene best plane (Fig. 2). Substitution of methanol for water molecule O4*W* results in the interruption of the helical chain of O—H···O hydrogen bonds (Fig. 4). We are presently checking whether cocrystallization of rifampicin with some other alcohols will result in similar structures.

Experimental

Rifampicin (1 mg, 0.001 mmol) was dissolved in a mixture of water (0.02 ml) and ethanol (0.10 ml). From this alcohol–water solution, red crystals of (I) suitable for X-ray analysis were obtained.

Crystal data

$C_{43}H_{58}N_4O_{12}\cdot 5H_2O$
$M_r = 913.01$
Orthorhombic, $P2_12_12_1$
$a = 13.8506$ (6) Å
$b = 17.3867$ (8) Å
$c = 19.7476$ (8) Å
$V = 4755.5$ (4) Å^3
$Z = 4$
Cu $K\alpha$ radiation
$\mu = 0.82$ mm^{-1}
$T = 130$ K
0.25 × 0.25 × 0.1 mm

Data collection

Oxford SuperNova diffractometer
Absorption correction: multi-scan (*CrysAlis PRO*; Agilent, 2010)
$T_{min} = 0.834$, $T_{max} = 1.000$
13055 measured reflections
4805 independent reflections
4531 reflections with $I > 2\sigma(I)$
$R_{int} = 0.029$

Refinement

$R[F^2 > 2\sigma(F^2)] = 0.048$
$wR(F^2) = 0.135$
$S = 1.06$
4805 reflections
597 parameters
H-atom parameters constrained
$\Delta\rho_{max} = 0.44$ e Å^{-3}
$\Delta\rho_{min} = -0.49$ e Å^{-3}

All H atoms were located in difference electron-density maps. Some water H atoms appeared as peaks of low density and hydrogen-bonding interactions were taken into account for their identification. Atoms H1O1 and H40N were placed in positions determined from a difference electron-density map and refined as riding, with their isotropic displacement parameters freely refined. H atoms of C—H

Table 1
Geometry of hydrogen bonds and other contacts (Å, °).

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
O1—H1O1···O8	1.00	1.59	2.499 (3)	148
O4—H1O4···O11	0.84	1.71	2.542 (3)	168
O21—H21O···O23	0.84	1.97	2.708 (3)	147
N2—H1N2···N38	0.88	2.24	2.706 (4)	113
O23—H23O···O4^{i}	0.84	1.96	2.783 (3)	165
N40—H40N···O1*W*^{ii}	1.06	1.71	2.769 (5)	170
O1*W*—H2*W*1···O27^{iii}	0.84	2.00	2.835 (5)	178
O2*W*—H2*W*2···O21	0.84	1.99	2.795 (4)	162
O3*W*—H1*W*3···O8^{iv}	0.84	1.95	2.784 (4)	170
O3*W*—H2*W*3···O11^{i}	0.84	1.85	2.680 (3)	170
O5*W*—H1*W*5···O15	0.84	2.00	2.841 (5)	178
O4*W*—H2*W*4···O1*W*	0.84	2.06	2.812 (6)	149
O1*W*—H1*W*1···O2*W*	0.84	1.96	2.703 (5)	147
O2*W*—H1*W*2···O3*W*	0.84	2.06	2.854 (5)	156
O4*W*—H1*W*4···O3*W*^{iv}	0.84	2.08	2.915 (6)	174
C31—H31*B*···O15^{v}	0.98	2.45	3.360 (4)	154
C36—H36*A*···O15^{i}	0.98	2.54	3.431 (5)	152

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

groups and the amide N—H group were placed at idealized positions and refined as riding, with C—H = 0.93–1.00 Å and N—H = 0.88 Å, and with U_{iso}(H) = 1.2U_{eq}(C), except for methyl groups, for which U_{iso}(H) = 1.5U_{eq}(C). In the water molecules, the O—H and H···H distances were initially restrained to 0.840 (1) and 1.35 (1) Å, respectively, and in the final cycles of refinement a riding model was applied with U_{iso}(H) = 1.2U_{eq}(O). The O—H bond lengths in the hydroxy groups (except O1—H1O1) were constrained to 0.84 Å and the U_{iso}(H) values were constrained to 1.2U_{eq}(O). In the absence of significant anomalous scattering effects, Friedel pairs were merged as equivalent data. The absolute structure is based on the known absolute configuration of rifampicin.

Data collection: *CrysAlis PRO* (Agilent, 2010); cell refinement: *CrysAlis PRO*; data reduction: *CrysAlis PRO*; program(s) used to solve structure: *SIR2004* (Burla *et al.*, 2005); program(s) used to refine structure: *SHELXL97* (Sheldrick, 2008); molecular graphics: *ORTEP-3 for Windows* (Farrugia, 1997) and *Mercury* (Macrae *et al.*, 2006); software used to prepare material for publication: *SHELXL97*.

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Supplementary data for this paper are available from the IUCr electronic archives (Reference: FA3275). Services for accessing these data are described at the back of the journal.

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