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THE CRYSTAL STRUCTURE AND QUANTUM MECHANICAL TREATMENT OF THE ANTI-CANCER AGENT FLAVOPIRIDOL (HYDROCHLORIDE) AND THE CHROMONE ALKALOID ROHITUKINE

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ABSTRACT

The characterisation of the solid-state crystal structure of the hydrochloric acid salt of anti-cancer agent *Flavopiridol* (*i.e.*, (–)-2-(2-chlorophenyl)-5,7-dihydroxy-8-[(3*S*,4*R*)-3-hydroxy-1-methyl-4-piperidinyl]-4*H*-1-benzopyran-4-one) is described. The title compound forms meta-stable X-ray quality crystals by slow evaporation of solutions of the material in aqueous methanol. The crystalline unit cell contains two organic cations, two formal chloride counterions and two molecules of methanol, one of which is replaced in 20% occupancy by a water molecule. The crystal form is of space group *P*1 with cell parameters a = 7.2014(10) Å, b = 12.0094(9) Å, c = 12.6581(14) Å, α = 89.146(4)°, β = 89.788(6)° and γ = 82.180(4)°. The unit cell volume is 1084.4(2)Å^3. The general structural features of individual (gas-phase) molecules of protonated Flavopiridol and the naturally occurring 2-methyl-[4*H*]-chromen-4-one analogue *Rohitukine* have been calculated by application of Density Functional Theory (DFT) at the B3LYP/6-31G* level of sophistication. These results are compared to the reported solid-state data of these two biologically relevant flavanoids.

INTRODUCTION

Flavanoids are a class of natural products that are widely distributed in the biosphere, particularly in the plant kingdom (Harborne & Mabry, 1982), as primarily secondary metabolites. Many flavanoids are, not surprisingly, biologically active and hence have been the subject of intense scrutiny by natural products chemists and the pharmaceutical industry (Eisnor et al., 2006; Joule et al., 1995). These investigations

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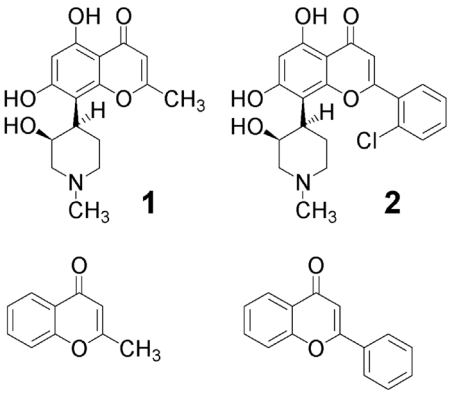
have led to the identification of a vast number of potential new sources of phytochemicals that have been used or are under development as both nutraceuticals and/or chemotherapy agents. In addition, novel structural motifs derived from natural products often serve as starting points or "leads" for compounds that are investigated for their clinical potential. Many years ago, the flavanoid compound dubbed *Rohitukine* (**1**: Scheme 1), which contains a 2-methyl-[4*H*]-chromen-4-one frag-



Scheme 1 Schematic representations of *Rohitukine* (**1**) and *Flavopiridol* (**2**). Below these pictorials are the parent structures of 2-methyl-[4*H*]-chromen-4-one and flavone: left and right, respectively.

ment, was isolated (Harmon et al., 1979) from the Asian plant *Amoora rohituka* (Syn. *Aphanamixis polystachya*) (meliaceae). This alkaloid incorporates both the aforementioned chromone skeleton, a common organic motif (Joule et al., 1995), and an unusual modified piperidinyl ring system. Rohitukine itself displays a range of biological activities including its ability to modulate immune response systems and act as an anti-inflammatory agent (Naik et al., 1998; Sedlacek et al., 1996). However, it is most noteworthy for its potent anti-cancer potential. Large quantities of **1** can be obtained from *Dysoxylum binectariferum* (Yang et al., 2004; Mohanakumara et al., 2010), which is currently the primary natural source of this chromone, although two species of *Schumanniophyton* also produce **1** (Houghton, 2002; Houghton & Hairong, 1987; Houghton & Woldemariam, 1993). Chemical modification of the basic structure of **1** has led to the synthesis of a more biologically active flavone (Harborne & Mabry, 1982) derivative given the name *Flavopiridol* (**2**: 2-[2-chlorophenyl]-5,7-dihydroxy-

8-[{3*S*,4*R*}-3-hydroxy-1-methyl-4-piperidinyl]-4*H*-1-benzopyran-4-one: Scheme 1). This compound has already entered Phase III clinical trials for applications against a number of classes of cancer including breast, colon and lung cancers, leukaemia and cancers of the head and neck (Billard et al., 2003; Carlson et al., 1996; Fischer & Lane, 2000; Kitada et al., 2000; Lin & Porcu, 2004; Patel et al., 1998; Wu et al., 2002). The mode(s) of action of **2** have been identified as primarily due to the ability of the compound to act as a selective kinase inhibitor and thus it represents the first such inhibitor to enter clinical trials (Bishop et al., 2001; Byrd et al., 1998; Carlson et al., 1996; Fabbro et al., 2002; Filigueira et al., 1996 & 2002; Huwe et al., 2003; Kitada et al., 2000; König et al., 1997; Kryŝtof & Strnad, 2003; Noble et al., 2004; Patel et al., 1998; Pepper et al., 2003; Sedlacek, 2001; Sedlacek et al., 1996; Senderowicz et al., 1999; Senderowicz & Sausville, 2000; Takada & Aggarwal, 2004; Wang & Ren, 2010). A brief report of the characterisation of **1**, *via* single crystal X-ray diffraction methods, has been previously reported (Yang et al., 2003) but details of the structure (bond lengths, bond angles, *etc*.) were not disclosed. Flavopiridol has not been investigated in this way. Neither **1** nor **2** have been structurally examined from a theoretical perspective by quantum mechanical methods. In this report, dedicated to the 150th anniversary of the *Nova Scotian Institute of Science*, we disclose the characterisation of the hydrochloric acid salt of **2** by X-ray diffraction methods and compare the solid-state structures of both **1** and **2** to those obtained by examining the molecules *via* quantum mechanical methods, specifically employing Density Functional Theory (DFT) at the B3LYP/6-31G* level of theory (Goodman, 1998; Koch & Holthausen, 2002; Sholl & Steckel, 2009).

METHODS

Flavopiridol hydrochloric acid salt (*i.e*., *Alvocidib*) was kindly supplied to the authors by Sanofi-Aventis, Inc. **Caution!** Flavopiridol is a potent biologically active agent and therefore should only be handled by qualified personnel using strict laboratory safety protocols. Crystals of the material were obtained by dissolving approximately 25 mg of the compound in methanol (~10 mL) and then allowing the resulting solution, contained in a small vial, to slowly evaporate under ambient conditions. Yellow rectangular shaped crystals were obtained after a period of about 7 days. These meta-stable crystals remain intact for a

period of about 6 weeks before returning to a powder form of (presumably) solvent-free material.

X-ray diffraction data was collected at –100°C on a Nonius Kappa CCD diffractometer, using the COLLECT program (Nonius, 1998). Cell refinement and data reductions used the programs DENZO and SCALEPACK (Otwinowski & Minor, 1997). SIR97 (Altomare et al., 1999) was used to solve the structure and SHELXL97 (Sheldrick, 2008) was used to refine the structure. ORTEP-3 for Windows (Farrugia, 1997) was used for molecular graphics (Figure 1) and PLATON (Spek, 2001) was used to prepare material for publication. H atoms were placed in calculated positions with U_{iso} constrained to be 1.2 times U_{eq} of the carrier atom for all hydrogen atoms. The structure solution has two cations and two chloride ions in the asymmetric unit. In addition, there are two solvent methanol molecules in the asymmetric unit, one with disorder. Twenty percent of one methanol is replaced by a water molecule. Modelling the disorder proved to be difficult because of the strong coupling between the occupancy factors and the thermal factors. In the end, the occupancy of water was set at 0.20 and the methanol at 0.80 to stabilize the refinement. The only B ALERT is for possible higher symmetry. This test does not consider the disordered atoms. Since one methanol is disordered with a water molecule and the other is not, higher symmetry is not possible. Crystallographic data (excluding structure factors) have been deposited in the *Cambridge Crystallographic Data Centre* as Supplementary publication No. CCDC 832180. Copies of these data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

Density Functional Theory was used for the quantum mechanical calculations employing the B3LYP/6-31G* level of theory (Becke, 1993; Lee et al., 1988); these data were obtained by using the SPARTAN 10.0 (Spartan, 2010) suite of programs. These calculations included neutral **1** and the cationic *N*-protonated form of Flavopiridol (*i.e.*, [**2+H**]$^+$). Zero point energy calculations were performed on the idealised structures to ensure that the data reflect true minima along the potential energy surface and hence no negative IR or Raman frequencies were calculated. Details of these data (including .mol files) are available from the authors on request.

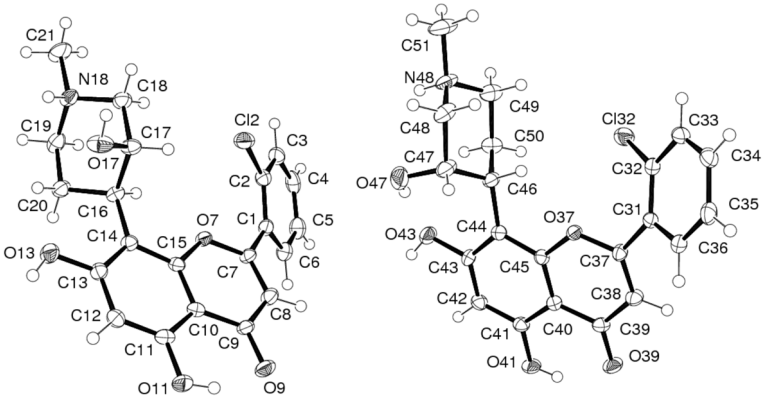


Fig 1 ORTEP representation of the two independent cations of [**2**+H] found in the unit cell; cation A (left) and cation B (right).

RESULTS AND DISCUSSION

The general crystal data parameters for the HCl salt of **2** (*i.e.*, [**2**+H]Cl) can be found in Table 1. A list of selected bond lengths, bond and torsion angles for [**2**+H]Cl, in addition to the computationally derived gas phase values of both **1** and cationic $[\mathbf{2}+H]^+$, are displayed in Table 2. Molecular representations (ORTEP) of the two independent [2+H] cations found within the unit cell are shown in Figure 1. In many respects, the solid-state structure of the cation of **2** is very similar to that described for other structurally characterised flavones with quite typical bond lengths and angles, specifically with respect to the benzopyrone and aromatic ring systems (Allen et al., 1987). 5-Hydroxyflavones, such as the title material, typically display intra-molecular *H*-bonding between the H atom on ring position-5 and the carbonyl O-atom (Chou et al., 2002; Krishnaiah et al., 2005; Parvez et al., 2001; Shoja 1989, 1990; Watson et al., 1991) and this certainly appears to be evident here (O9H•••O = 1.856Å). The benzopyrone ring is essentially planar with a torsion angle of less than 3°, similar to other flavones that have been previously reported (*e.g.*, Krishnaiah et al., 2005) and this also appears to be the case for Rohitukine (Yang et al., 2003). For simplicity, direct comparisons will be noted for unit cell Molecule A only. Details of Molecule B can be found in the appropriate .cif file and/or details noted in Table 2. The overall crystal motif reveals considerable

Table 1 General crystal data for compound $(2+HCl)_2 \bullet (MeOH)_{1.80} \bullet (H_2O)_{0.20}$

Parameter:	**$(2+HCl)_2 \bullet (MeOH)_{1.80} \bullet (H_2O)_{0.20}$**
Formula	$C_{43.80}H_{49.60}N_2O_{12}Cl_4$
fw	937.85
Crystal size (mm)	0.25 × 0.20 × 0.13
a (Å)	7.2014(10)
b (Å)	12.0094(9)
c (Å)	12.6581(14)
α (°)	89.146(4)
β (°)	89.788(6)
γ (°)	82.180(4)
V (Å^3)	1084.4(2)
D_{calc} (g/cm^3)	1.436
Crystal system; space group	Triclinic; P1
Z	1
F(000)	490.4
T (K)	173(2)
Absorption coefficient (mm^{-1})	0.339
2θ range (°)	2.86 – 27.63
Limiting indices	$-9 \le h \le +9$; $-15 \le k \le +15$; $-16 \le l \le +16$
Reflections collected	15962
Reflections unique	8544 [R(int) = 0.0414]
Reflections I > 2σ(I)	8544
Restraints / Parameters	3 / 571
GOF on F^2	1.055
Final R indices I > 2σ(I)	$R_1 = 0.0422$; $wR_2 = 0.0911$
R indices (all data)	$R_1 = 0.0522$; $wR_2 = 0.0987$
$\varrho_{min,max}$ (e·Å^3)	0.269, –0.284
Abs. Structure Parameter	–0.01(4)
λ (Å)	0.71073 Mo Kα

Table 2 Selected bond lengths (Å), bond and torsion angles (°) measured for [**2**+HCl]$_2$•(MeOH)$_{1.80}$•(H$_2$O)$_{0.20}$ and calculated (DFT: B3LYP/6-31G*) for **1** and [**2**+H]$^+$. Estimated standard deviations are shown in parentheses.

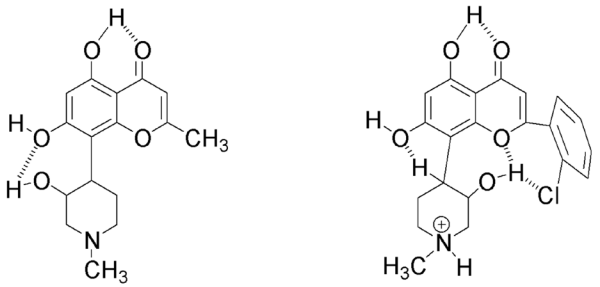
Value	1 (calc.)	[2+H]$^+$.(calc.)	[2+H]Cl•(observed)[a]
C=O (Å)	1.251	1.243	1.263(4); 1.269(3)
C=C (Å)	1.351	1.346	1.343(4); 1.358(4)
$C_{aromatic}$-OH (Å)	1.339; 1.359	1.330; 1.363	1.360(3); 1.364(4) 1.358(3); 1.368(3)
C-Cl (Å)	n/a	1.761	1.737(3); 1.741(3)
C=C-CH_3 (°)	126.2	n/a	n/a
C=C-C_{phenyl} (°)	n/a	124.84	125.4(3); 125.6(3)
C-CH-CH-OH (°)	56.13	-77.10	-66.4(3); -63.6(4)
C=C-C-CCl (°)	n/a	106.42	-143.0(3); 141.3(3)

[a] Entries for Molecule A (see text) are listed first.

intermolecular *H*-bonding aspects, specifically the 7-OH group with a formal chloride anion (O13H•••Cl = 2.23Å). The N-H functionality is also in close proximity to one of the lattice methanol O atoms (N18H•••O = 1.96Å). The chlorophenyl group is oriented out of the plane of the benzopyrone ring by about 41°; a property which decreases the contact distance between the Cl atom and the H on piperidinyl ring position 3' (C17H•••Cl = 2.86Å). Other flavones (Hall et al., 2001; Waller et al., 2005), including 2'-substituted examples, also display such large angles (Chou et al., 2002; Shoja, 1989; Ting et al., 1972) and indeed a value of 62° has been reported for 6-hydroxy-2',3'-dimethoxyflavone (Wallet et al., 1993).

Flavopiridol hydrochloride obviously demonstrates considerable inter- and intra-molecular *H*-bonding, as detailed above, in the solid-state. The obvious low volatility of this salt negates one's ability to also examine this material in the gas-phase to evaluate any *H*-bonding facets. However, the examination of compounds from a theoretical perspective allows one to probe the structural aspects of such species in the hypothetical gas-phase (*i.e.*, a single isolated molecule). Of the plethora of computational methods that can be used in this regard, Density Functional Theory (DFT) has become a widely employed and powerful tool to examine molecules and molecular fragments from a theoretical point of view in the gas-phase, solution and indeed even in network solids (Goodman, 1998; Koch & Holthausen, 2002; Sholl

& Steckel, 2009). An examination of both **1** and [**2**+H]$^+$ was therefore carried out using DFT to examine the overall structural properties and attempt to draw some conclusions about possible gas-phase structures of these two species. Selected calculated bond lengths and bond and torsion angles can be found in Table 2. As expected (Chojnacka et al., 2011), the DFT calculations do closely parallel the solid-state structures of the two materials. As details of the bond lengths and angles for the crystalline state of **1** do not appear in the literature (Yang et al., 2003), a comparison of calculated **1** to that of [**2**+H]$^+$ depicts a reasonable structural similarity between the two species (Table 2). As inter-molecular *H*-bonding cannot be involved here, this restricts the attractive forces to those of an intra-molecular nature. A skeletal diagram indicating the calculated *H*-bonding aspects (dashed line 'bonds') is shown in Scheme 2. The calculated Flavopiridol cation displays lesser rotation of the aromatic group with respect to that of the benzopyrone ring (calc. 106°; found 141°) and this serves to facilitate *H*-bonding between the 3'-OH (piperdinyl) group and the chlorine atom (calc. O17H•••Cl = 2.65Å). Obviously, this latter result causes considerable rotation of the piperdinyl ring and this again strengthens intra-molecular *H*-bonding, in this case between the same –OH and the benzopyrone ether O atom (O17H•••O7 = 2.05Å). These latter aspects do not appear for **1** but instead strong interactions between the piperonyl OH and the benzopyrone 7'-OH position is observed (O17H•••O = 1.88Å: Table 2; Scheme 2). Not surprisingly, both calculated structures include close contacts between the H atom on ring position-5 and the



Scheme 2 Schematic representation of the H-bonds (dashed lines) calculated for gas-phase 1 and the Flavopiridol cation ([**2**+H]$^+$). In both cases, stereochemical bond descriptors have been removed for clarity.

carbonyl O-atom although a slight over estimation of this strength is noted (calc. O11H•••O9 = 1.69Å for **1** and 1.70Å for [**2**+H]$^+$). The C=O (**1**: 1.25Å; **2**: 1.24Å) and C-Cl bonds ([**2**+H]$^+$) are well-estimated in both cases (**2**: C-Cl = 1.76Å). The calculated UV-Visible absorptions are also estimated with fair accuracy for both **1** (λ_{max} [calc.]: 241 nm; λ_{max}: [observed]: 252 nm) and [**2**+H]Cl (λ_{max} [calc.]: 247 nm; λ_{max}: [observed: aqueous]: 269 nm), despite the molecular rearrangements noted above for gas-phase calculations (Sedlacek et al., 1996; Tang et al., 2004; Yang et al., 2009).

CONCLUSIONS

The solid-state crystal structure of Flavopiridol hydrochloride, in the form of meta-stable crystals containing both methanol and water molecules, has been detailed. This compound has features similar to other related flavanoids that have been characterised in the solid-state such as Rohitukine. This latter material and the cationic component of the title compound have been further examined from a theoretical perspective by Density Functional Theory and these results suggest a modified pattern of *H*-bonding for individual gas-phase molecules.

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