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Short communication

Physico-chemical and structural characterization of diacerhein

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ABSTRACT

The physico-chemical and structural characterization of diacerhein, an anthraquinone with antiinflammatory activity, employed in the symptomatic treatment of inflammatory osteoarthritis, is reported. The combined use of FT-IR, DSC, X-ray powder and single-crystal diffraction has provided a valuable tool to fully characterize the title compound. The X-ray diffraction study has revealed the existence of hydrogen-bond assisted tight packing of the quasi-planar molecules in the solid. The collected results are intended to implement the information required for the compilation of drug master files.

1. Introduction

Diacerhein, 4,5-diacetoxy-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid (Fig. 1), is an anthraquinone with antiinflammatory properties previously evidenced in experimental models [1,2]. Clinical studies suggest that diacerhein provides a slowly acting symptomatic treatment of inflammatory osteoarthritis [3,4]. In humans and animals diacerhein is completely metabolized to rhein [5], which affects cells [6] reducing the production of superoxide anions and the chemotaxis, i.e. the migration and phagocytic activity of neutrophils and macrophages [7]. Diacerhein has been proved to inhibit the synthesis and activity of proinflammatory cytokines [8–11]. In a recent report [12], two mechanisms of action have been observed: in vitro inhibition of the synthesis of interleukin-1 (IL-1), the main cytokine involved in cartilage destruction, and activity on the synthesis of proteoglycans and hyaluronic acid, the principal component of cartilage. Moreover, Cruz and Pastrak have reported the use of rhein or diacerhein to treat and prevent vascular diseases [13].

Diacerhein is readily obtained in few synthetic steps from the naturally occurring glucopyranoside aloin [14,15].

In spite of the longstanding commercial distribution of diacerhein in the solid state as oral tablets, reliable crystallographic information on the compound is still lacking. This is a drawback when drug master files are to be compiled. In particular, detailed structural information is a powerful aid when the ubiquitous and vexing problem of polymorphism of drugs in the solid state is encountered. In this view, the combination of single-crystal and powder diffraction data, when available, provides the definitive assessment of the actual form of the solid material being investigated [16,17].

2. Experimental

2.1. Materials

Diacerhein standard material of pharmaceutical purity grade, was kindly provided by SIMS (Reggello, Firenze, Italy).

The solvents used were of analytical grade.

2.2. Preparation of the samples

Single crystals of diacerhein, suitable for X-ray single-crystal diffraction (XRSCD) investigation, could be obtained by slow evaporation both from THF and MeOH solutions.

2.3. Fourier transform infrared spectroscopy (FT-IR)

Spectra were recorded on a PerkinElmer Mod. Spectrum 1000 FT-IR spectrophotometer, equipped with a deuterium triglycine sulfate (DTGS) detector. Setting parameters: resolution 4 cm^{-1}; apodization weak. The data region was 4000–700 cm^{-1} and the

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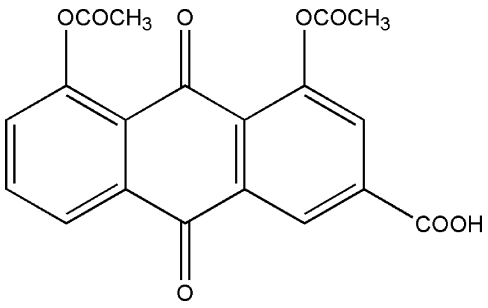

Fig. 1. Diacerhein.

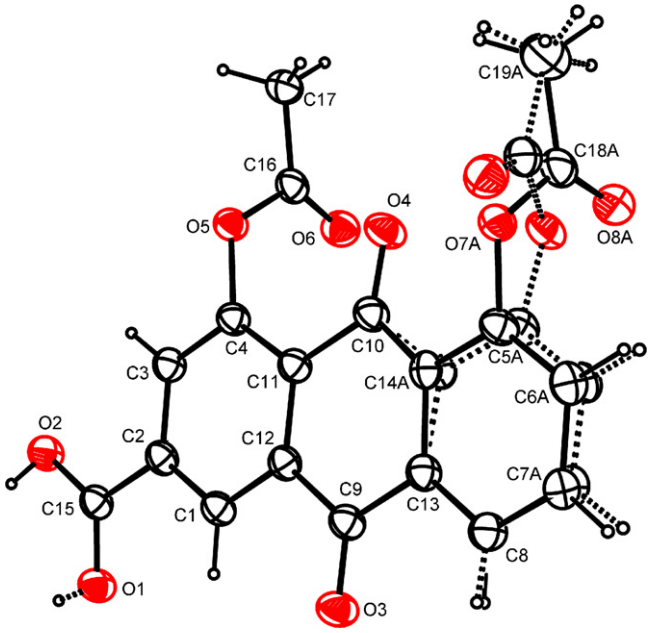

Fig. 2. A view of the molecule of diacerhein. Displacement ellipsoids are drawn at the 30% probability level. Dashed bonds denote the minor fractions of the parts (acetyloxy and carboxyl group) affected by disorder. Only the sites of the major fraction of the disordered acetyloxy group are labelled, for clarity.

number of scans per spectrum 60. Spectra were obtained in the transmission mode in nujol and in the HATR mode (Horizontal Attenuated Total Reflectance, Pike Miracle ATR Accessory).

2.4. XRSCD

Single-crystal X-ray diffraction data were collected at 173(2) K with an Oxford Diffraction Xcalibur PX Ultra CCD diffractometer, using Oxford Diffraction software [18] and Cu Kα radiation (λ = 1.5418 Å).

Crystal data: $C_{19}H_{12}O_8$, M_w = 368.29, triclinic, space group $P\bar{1}$, a = 5.2638(2) Å, b = 9.0648(3) Å, c = 17.0840(6) Å, α = 79.845(3)°, β = 82.748(3)°, γ = 74.066(3)°, V = 768.96(5) Å^3, Z = 2, D_c = 1.591 g cm^{-3}, pale yellow, flat prism, 0.02 mm × 0.20 mm × 0.40 mm, μ = 1.08 mm^{-1}.

In view of the shape of the crystal, a correction for absorption was applied to the data with the ABSPACK routine of the *CrisAlisPro* suite of programs [18] (0.532–1.000 transmission factors range). The structure was solved by direct methods with SIR-97 [19] and was completed and refined with SHELXL-97 [20]. There is one molecule of the compound in the asymmetric unit of the triclinic unit cell, and it is affected by twofold orientational disorder of the acetyloxy substituent at C5 (Fig. 2), with small consequences on the positions of the nearest carbon atoms of the molecular backbone. The disordered part of the molecule was refined as two complementary fractions, with geometrical restraints. An additional small effect of disorder was found for the carboxylic group, where the hydrogen atom, involved in hydrogen bonding between carboxyls (see below), was almost equally distributed between the two oxygen sites, with small preference for the O2 position [0.58(4) population parameter for the O2—H bond, compared to the complementary value for the O1—H bond]. Of the 13491 measured reflections 3025 were independent, of which 2630 had $I > 2\sigma(I)$. In the final refinement cycles, on F^2 (325 parameters, 48 restraints) all non-hydrogen atoms were refined anisotropically and hydrogen atoms were in geometrically generated positions. The final values of the R indices were: R_1 = 0.051, wR_2 = 0.143 (for reflections with $I > 2\sigma(I)$), R_1 = 0.058, wR_2 = 0.157 (all data). The highest/lowest features in the final ΔF map were 0.23/−0.24 e Å^{-3}. Programs used for crystallographic calculations included PARST [21] for the analysis of geometry and ORTEP [22] and PLATON [23] for drawings. Complete results from the crystallographic analysis have been deposited as a CIF file with the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033; e-mail:

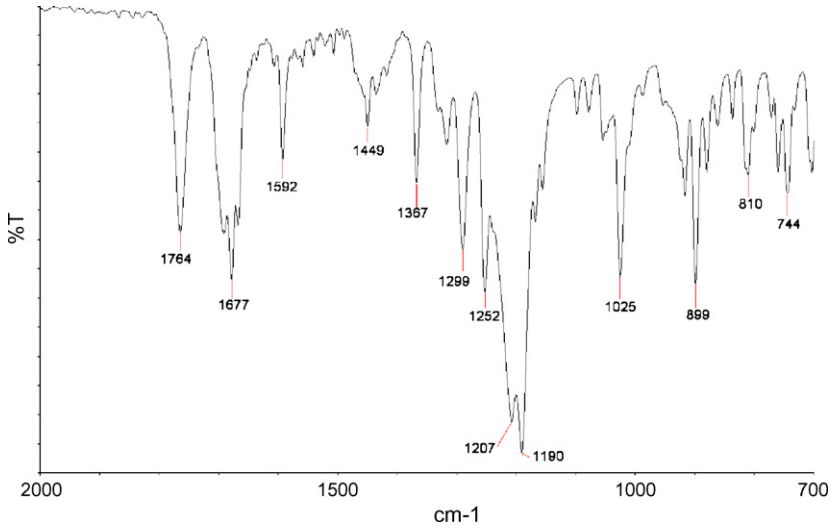

Fig. 3. FT-IR spectrum of diacerhein (HATR mode); fingerprint region.

Table 1
Selected values of bond distances (Å), bond angles (°) and torsion angles (°) in the diacerhein molecule[a].

C1—C2	1.388(2)	C8—C7B	1.39(1)
C2—C3	1.393(2)	C6A—C7A	1.38(1)
C3—C4	1.380(2)	C6B—C7B	1.38(1)
C4—C11	1.405(2)	C5A—C6A	1.38(1)
C11—C12	1.401(2)	C5B—C6B	1.37(1)
C1—C12	1.400(2)	C5A—C14A	1.40(1)
C9—C12	1.488(2)	C5B—C14B	1.40(1)
C9—C13	1.490(2)	C4—O5	1.391(2)
C13—C14A	1.405(9)	C5A—O7A	1.394(8)
C13—C14B	1.415(9)	C5B—O7B	1.390(8)
C10—C14A	1.506(8)	C9—O3	1.214(2)
C10—C14B	1.492(9)	C10—O4	1.212(2)
C10—C11	1.506(2)	C2—C15	1.495(2)
C8—C13	1.390(2)	C15—O1	1.264(2)
C8—C7A	1.40(1)	C15—O2	1.270(2)
C3—C4—O5	116.4(1)	C6B—C5B—O7B	117.5(9)
C11—C4—O5	122.1(1)	C14A—C5A—O7A	118.5(7)
C6A—C5A—O7A	118.0(8)	C14B—C5B—O7B	121.1(7)
C2—C3—C4—O5	−174.6(1)	C13—C14A—C5A—O7A	−171.6(6)
C12—C11—C10—O4	173.4(2)	C13—C14B—C5B—O7B	174.8(7)
C11—C12—C9—O3	178.7(2)	C1—C2—C15—O1	−6.8(2)

[a] Atoms belonging to the two fractions of the disordered part of the molecule are respectively denoted by the A and B labels.

deposit@ccdc.cam.ac.uk) and are available on request quoting the deposition number CCDC 711847.

2.5. X-ray powder diffraction (XRPD)

The diffraction pattern from a sample of diacerhein standard was collected on a Bruker D8-Advance powder diffractometer, in the Bragg–Brentano θ–θ geometry, using Cu Kα radiation and working at 40 kV and 40 mA. The Sol-X® solid state Si(Li) detector was used; 1.0 mm divergence and scatter slits and a 0.1 mm receiving slit were used. The diffraction pattern was collected in the 3–50° 2θ range, with 0.02° steps and 1 s/step counting time. The comparison powder pattern was generated with parameters from the present single-crystal X-ray structure of diacerhein, using the CCDC program Mercury [24].

2.6. Thermal analysis

Differential scanning calorimetry (DSC) was performed using a PerkinElmer mod. Pyris 1 instrument and Pyris software for Windows. Data were recorded using a heating rate of 20 °C min^{-1}.

3. Results and discussion

3.1. FT-IR

The HATR-FT-IR spectrum in the 2000–700 cm^{-1} region, of the diacerhein standard crystallized from THF, is reported in Fig. 3. FT-IR analyses were performed in the reflectance mode to avoid nujol interference and modifications that might take place by grinding for sample preparation. In the present case no distinctive changes due to vigorous grinding were revealed by comparison of the FT-IR spectra with the exception of the C—O—C peak resolution (1207 and 1190 cm^{-1} by HATR vs. 1208 cm^{-1} by nujol).

3.2. X-ray single-crystal diffraction

Selected values of bond distances and angles in the symmetry-independent molecule in the structure of diacerhein are listed in Table 1. The molecule is affected by twofold orientational disorder of the acetyloxy substituent at C5 (Fig. 2). Besides giving rise to the existence of two conformers in the structure, according to the orientation of the acetyloxy group [conformers A and B hereafter, respectively with 0.525(3) and 0.475(3) population parameters], such disordered orientation also causes small splittings of the positions of the nearest carbon atoms of the benzene ring to which the above substituent is attached. These features were found for all samples investigated, irrespectively of significant differences in the crystallization conditions. The disorder, on the other hand, does not prevent from discussing some geometrical features, which are worth of note. The molecular backbone is only approximately planar, with a 6.0(4)° dihedral angle between the planes through the benzene rings of conformer A and a 5.5(4)° angle for conformer B. All of the three oxygen atoms bound to one side of the group of fused rings deviate from the plane of the respective ring [e.g., the C4—O5 bond forms a 5.3(1)° angle with the plane of the benzene ring from which it departs and comparable deviations are exhibited by the fractional C5n—O7n (n = A, B) bonds]. Moreover, the C4—O5 bond and the two fractions of the C5—O7 one are tilted sideways, by ca. 3°, away from the central carbonyl bond. These features appear to be determined by steric crowding in the region of the three vicinal C—O bonds. Deviations of the molecule from planarity are common among similarly substituted anthraquinones [25–29], whereas inward bending of the lateral C—O bonds, opposite to that found for diacerhein, is often found [25,26,29], this being apparently due to the detailed shape and orientation of the substituents at C4 and C5 and/or to repulsions with additional substituents, not present in diacerhein. It

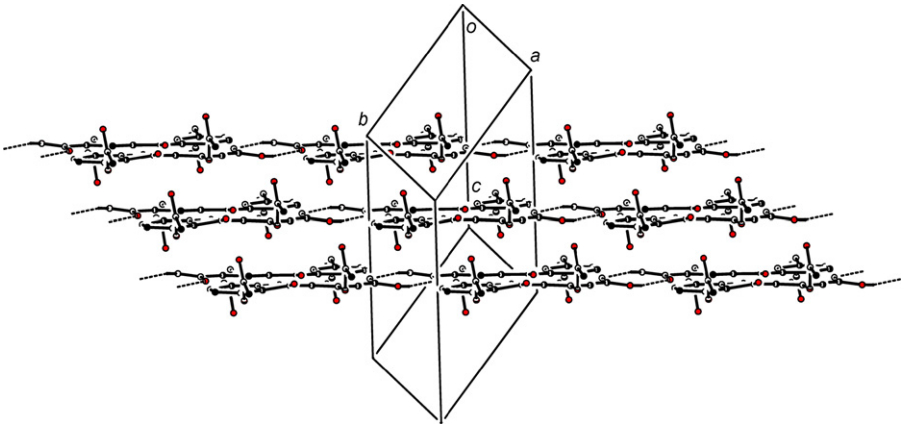

Fig. 4. Representation of the conventional, O—H···O, and non-conventional, C—H···O, hydrogen bonds along one of the ribbons characterizing the crystal structure of diacerhein. Only the major fraction of the disordered acetyloxy group and only one of the disordered arrangements of the hydrogen bonds between carboxyl groups are shown for clarity. Symmetry codes: (i) $-x-1, -y+2, -z+1$; (ii) $-x+1, -y+1, -z+1$; (iii) $x+2, y-1, z$.

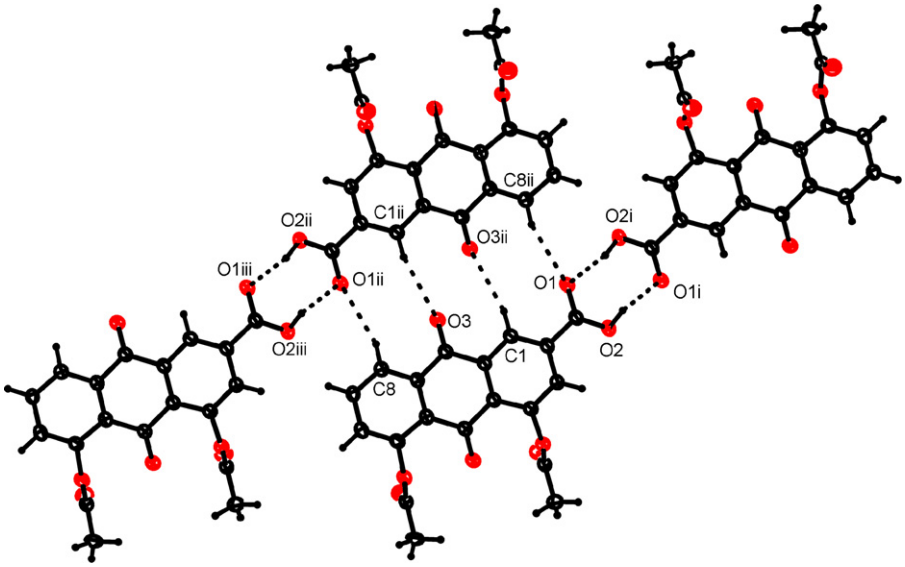

Fig. 5. A view of the crystal packing in the structure of diacerhein, showing parts of the ribbons of hydrogen-bonded molecules belonging to three adjacent layers. Only the carboxyl hydrogen atom and only one of the arrangements of each disordered part are shown for clarity.

should also be noted that when inward bending of the lateral C—O bonds occurs, this is generally accompanied by a significant deviation of the interposed carbonyl bond from the overall molecular plane [25,26,29].

The diacerhein molecules in the structure are arranged in tightly packed planes, formed in turn by ribbons of hydrogen-bonded molecules. There are centrosymmetric pairs of molecules head-on linked by "conventional" O—H···O hydrogen bonds, through their carboxyl groups. In addition, each molecule forms a "comb-like" set of four "non-conventional" C—H···O hydrogen bond interactions with another molecule, related to the former by a distinct inversion centre, giving rise in this way to a ribbon of molecules (Fig. 4). The ribbons are arranged in planes (Fig. 5) among which contacts as short as the 3.294(2) Å O5···C13^{i} one (symmetry code: $i = 1 - x, y, z$) are established. It appears that these structural features should not be ignored when considering the physico-chemical properties of the solid material in the present form.

3.3. X-ray powder diffraction

The X-ray powder diffraction pattern computationally generated using the parameters from the single-crystal structural study agrees satisfactorily with the experimental pattern obtained for diacerhein in microcrystalline form (Fig. 6). In particular, there is good agreement between the two patterns as far as the peak positions are concerned; the agreement between peak intensities is only fair, but it is well known that this aspect may be strongly influenced by the experimental conditions, whereas peak positions are related to the structural parameters. Therefore, it may be safely assumed that the material in microcrystalline form is isostructural with the crystals grown for the complete X-ray analysis. Moreover, in view of the close packing of layers of ribbons in the structure, it is unlikely that interposed solvent molecules are present in the starting material.

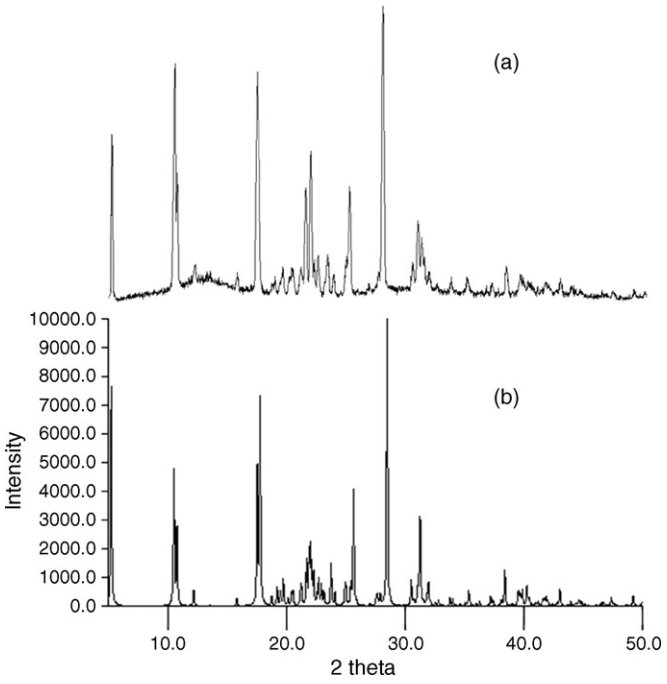

Fig. 6. The experimentally recorded (a) and computationally generated (b) X-ray powder diffraction spectra of diacerhein.

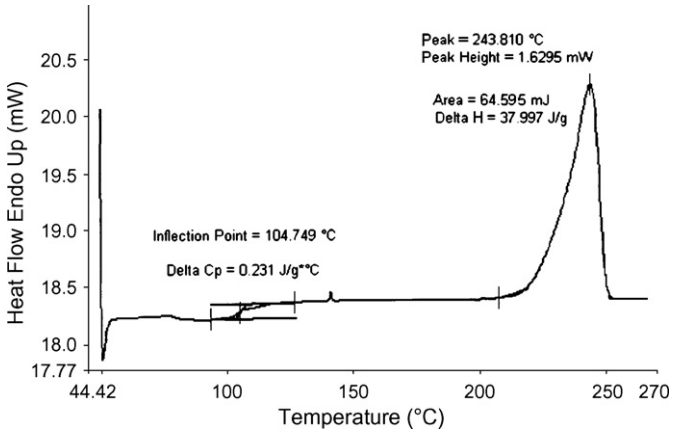

Fig. 7. Diacerhein DSC trace at 20 °C min^{-1}.

3.4. Thermal analysis

The DSC curve, in the 50–270 °C range at 20 °C min^{-1} heating rate, shows only one endotherm of fusion. Onset 218 °C (Fig. 7).

4. Conclusions

The physico-chemical and structural characterization of diacerhein, has been attained by the combined use of FT-IR, DSC, X-ray powder and single-crystal diffraction. The X-ray diffraction study has revealed the presence of tightly packed layers of molecules in the solid, formed in turn by parallel ribbons along which strong hydrogen bond interactions are established. The close similarity between the experimental and calculated X-ray powder diffraction spectra allows to assign the above type of arrangement to the material in its standard form. The collected data have provided a valuable tool to fully describe the title compound and are intended, inter-alia, to implement the information required for compilation of drug master files.

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