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Hirshfeld Surfaces Analysis of Intermolecular Interaction in the Series of Steroid Hormone Molecular Crystals

In this work Hirshfeld surface analysis is performed on the crystallographic-characterized crystal cells of the progesterone, 17α-hydroxyprogesterone, and testosterone hormones. The Hirshfeld surfaces are mapped for a detailed visualization of the electron density distribution around a molecule to understand the atomic-pair close contacts and interaction types within a crystal structure of the studied hormones. The intermolecular forces, including Van der Waals forces, hydrogen bonding, and C–H···π interactions, play essential roles in determining the supramolecular arrangement of all the three molecules in their crystals. These forces contribute to the cohesion, stability, and structural organization of the crystals, ultimately influencing their properties and behaviour in various applications. Two-dimensional fingerprint plots with detailed information about the contribution of each contact to the total Hirschfeld surface allowed to provide a visual representation of the relative importance (in %) of identified intermolecular O···H/H···O, C···H/H···C, H···H interactions within a crystal structure of the studied hormones in the context of "molecule-substance" relations.

Keywords: sex hormones; progesterone; 17α-hydroxyprogesterone; testosterone; intermolecular interactions; X-ray analysis; Hirshfeld surface analysis; fingerprints; hydrogen bonds.

Introduction

The most studied sex hormones, such as progesterone, 17α-hydroxyprogesterone, and testosterone ensure a stable development and functioning of the human body according to the male or female type. They play a crucial role in the maintenance of the male and female reproductive systems, as well as influencing various physiological processes throughout the human body. The delicate balance of these sex hormones is essential for the proper functioning and development of the human organism. Any level disruption or imbalance can result in various issues, affecting both reproductive and overall health. Hormonal regulation is a complex process involving feedback loops between the brain, gonads, and other endocrine glands. In a biochemical classification, progesterone, 17α-hydroxyprogesterone, and testosterone are steroids, as their structures are based on the cyclopentanoperhydrophenanthrene (sterane) cycle. The steroid nucleus, sterane, comprises three cyclohexane rings (A, B, and C) and one cyclopentane ring D (Fig. 1). This common structural motif imparts distinctive biochemical and physiological properties to the steroids and provide the lipophilicity that enables their traverse of cell membranes. This facilitates their interaction with intracellular receptors, initiating a cascade of events that ultimately modulate gene expression and influence various physiological processes. While these steroids share a common structural foundation, their specific functions and regulatory roles in the body are diverse. This specificity and diversity are determined to some extent by in-

termolecular contacts including local areas of van-der-Waals interactions and low-frequency vibration energy flow [1–4].

Progesterone, a C-21 steroid, plays a crucial role in the female reproductive system. It is the main hormone of pregnancy. The effect of progesterone is realized through its receptors where the C3-keto-group and a double bond between the C4 and C5 carbon atoms of cycle A are important features (Fig. 1) [1–5]. 17α-Hydroxyprogesterone, an intermediate in the synthesis of various steroid hormones, is particularly involved in the production of cortisol and androgens. It serves as a precursor in the biosynthetic pathways that lead to the formation of these important hormones. It differs from progesterone by the presence of an additional hydroxyl radical near the 17th carbon atom in the molecular skeleton (Fig. 1) [5].

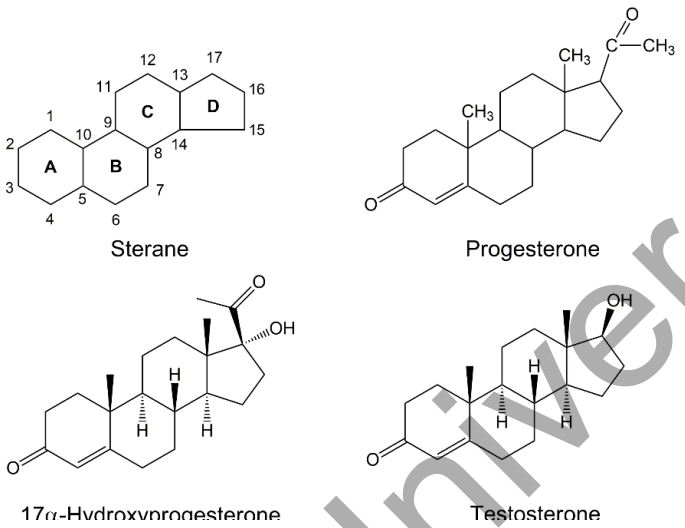


Figure 1. Chemical structures of sterane, progesterone, 17α-hydroxyprogesterone and testosterone

Testosterone, a C-19 steroid and the primary male sex hormone responsible for the development of male sexual characteristics [6–8]. This hormone is from the androstane class containing a ketone and a hydroxyl group at the C3 and C17 positions, respectively. An adult man's testosterone level is 7-8 times higher than that of an adult woman. Because the testosterone levels naturally decline as men age, the synthetic testosterone is sometimes used to counteract testosterone deficiency, and it is also used illegally to improve muscle mass and performance in athletes, a practice known as doping. The World Anti-Doping Agency (WADA) included it in the S1 Schedule of anabolic agents.

Analysis of the structure and spectral characteristics of hormones is important for gaining insights into the specificity of their interactions with corresponding receptors [4]. Quantum-chemical calculations of the geometry, electronic structure, and Raman spectrum of the progesterone molecule are presented in Ref. [9]. Intermolecular interactions are important for the formation of crystal packing, molecular recognition, and the formation of supramolecular structures [10]. Intermolecular interactions in crystals of progesterone, 17α-hydroxyprogesterone, and testosterone were investigated based on Kohn-Sham orbitals using Bader's topological theory [11]. To analyze the function of the electron density distribution, an additive scheme of breaking the crystal packing of hormones into six dimer pairs was used. Analysis of the electron density distribution function in progesterone, 17α-hydroxyprogesterone, and testosterone dimers revealed several hydrogen bonds O···H and non-valent bonds H···H, C···H, which stabilize the spatial structure of dimers. The total energy of all non-valent intermolecular bonds in the 17α-hydroxyprogesterone tetramer is equal to –19.78 kcal/mol, which significantly exceeds the energy of the crystal packing of progesterone (–9.67 kcal/mol) and testosterone (–10.62 kcal/mol) [12]. A drawback of the technique used in Ref. [9] is that it neglects the influence of neighbouring molecules on the electron density distribution in a particular dimer.

In this study, the analysis of intermolecular interactions in the crystal packings of progesterone, 17α-hydroxyprogesterone, and testosterone was carried out using the Hirshfeld surface method, which has been widely used in recent years as a means to quantify and visualize various types of intermolecular interactions in molecular crystals [13–17]. Analysis of the three-dimensional (3D) Hirschfeld surfaces and two-dimensional (2D) fingerprint plots provides details of the packing behaviour of molecular crystals and allow

us to determine the contribution of each type of non-valence links to the overall Hirschfeld surface of all intermolecular interactions in the crystal cell of the corresponding hormone.

Computational Details

Hirschfeld surface (HS) analysis was performed using CIF files of the X-ray structures of progesterone, 17α-hydroxyprogesterone, and testosterone. The studied hormones have an orthorhombic crystal packing and belong to the $P2_12_12_1$ symmetry space group [18–21]. Crystal packing parameters for the studied hormones were taken from crystallographic data deposited in the Cambridge Crystallographic Data Centre with the corresponding CCDC deposition numbers: 228768 (progesterone), 1178343 (17α-hydroxyprogesterone), 1269495 (testosterone).

The method of Hirschfeld surfaces is implemented in the CrystalExplorer 21.5 software package with a very high resolution [22, 23]. The analysis is visualized by the normalized contact distance (d_{norm}), which is defined in terms of d_e (the nearest external distance), d_i (the nearest internal distance), and the van der Waals (*vdW*) radii of the two atoms on the surface, and is calculated by the following equation [17]:

$$d_{norm} = \frac{d_i - r_i^{vdW}}{r_i^{vdW}} + \frac{d_e - r_e^{vdW}}{r_e^{vdW}},$$

where r_i^{vdW} and r_e^{vdW} are the van der Waals radii of the corresponding atoms, internal and external to the surface, respectively.

For a geometric representation of intermolecular interactions, the Hirshfeld surface is colored in red-white-blue colors depending on the d_{norm} value. The contacts with distances equal to the sum of the *vdW* radii are shown in white (moderate magnitude of intermolecular interactions), and the contacts with distances shorter or longer than the *vdW* radii are shown in red and blue, respectively (strong and weak magnitude of intermolecular interactions).

The d_{norm} decorated HSs are mainly discussed concerning the corresponding fingerprint plots. The fingerprint plot is a 2D diagram derived from the HS and shows the frequency of occurrence of each d_e and d_i combination on the surface. Each unique d_e *vs* d_i combination in the fingerprint plot is interpreted as representing a specific type of intermolecular interaction.

Previously, we used Hirschfeld surface analysis to study intermolecular interactions in cathinone crystal drugs and proved it to be an effective and versatile approach in forensic researches [24–26].

Results and Discussion

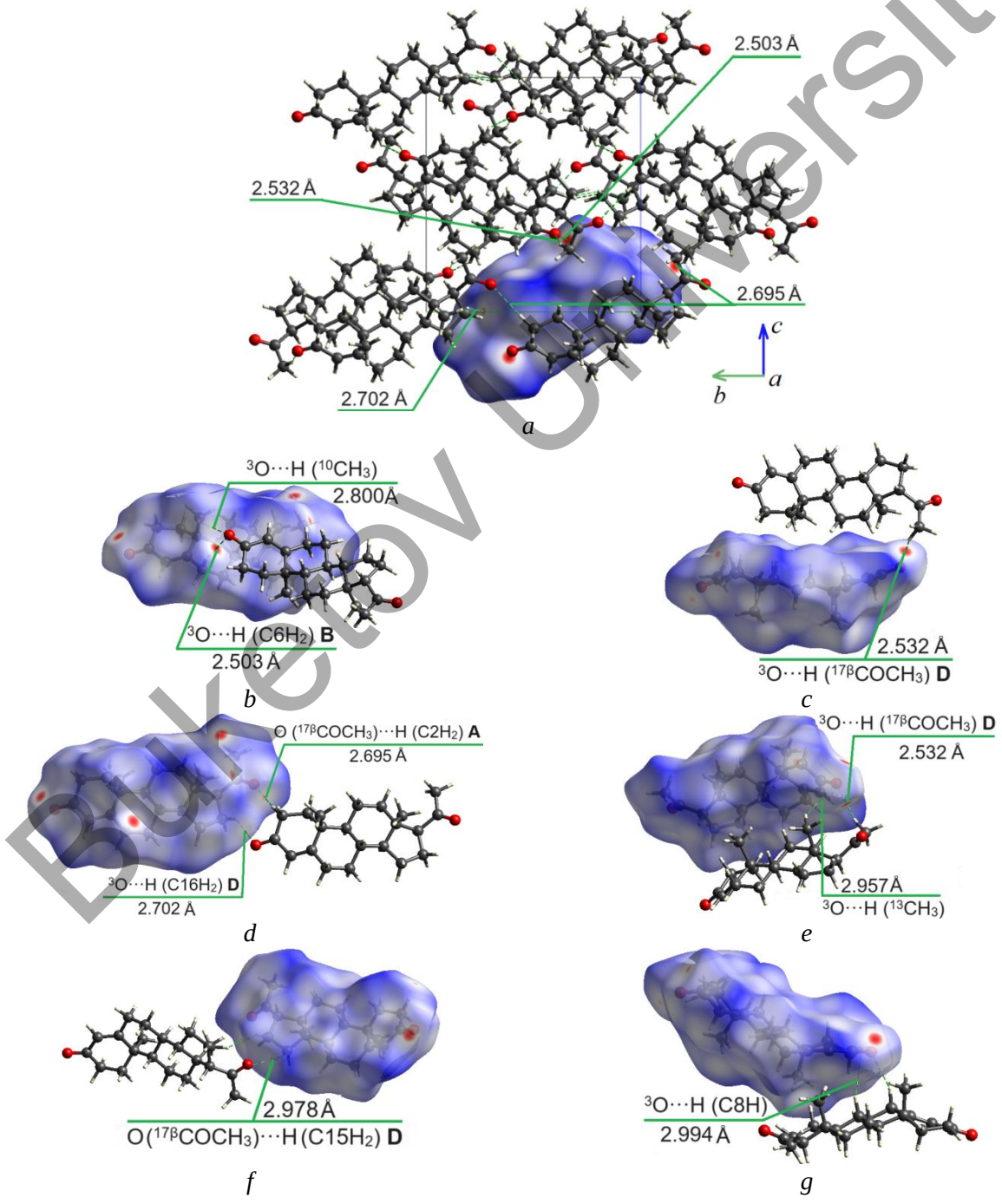
Selected intermolecular distances O···H in the crystalline cells of progesterone, 17α-hydroxyprogesterone and testosterone compounds are listed in Table (Here ^{3}O means, for example, the oxygen atom in position 3 of the sterane cycle in Figure 1).

T a b l e

Selected intermolecular O···H distances (*d*) in crystalline cells of progesterone, 17α-hydroxyprogesterone, and testosterone according to X-ray data

Parameter	*d*, Å	Parameter	*d*, Å
Progesterone			
^{3}O···H (C6H$_2$) **B**	2.503	^{3}O···H (^{13}CH$_3$)	2.957
^{3}O···H ($^{17\beta}$COCH$_3$) **D**	2.532	O ($^{17\beta}$COCH$_3$)···H (C15H$_2$) **D**	2.978
O ($^{17\beta}$COCH$_3$)···H (C2H$_2$) **A**	2.695	^{3}O···H (C8H)	2.994
^{3}O···H (C16H$_2$) **D**	2.702	O ($^{17\beta}$COCH$_3$)···H (C16H$_2$) **D**	3.100
^{3}O···H (^{10}CH$_3$)	2.800	O ($^{17\beta}$COCH$_3$)···H (C6H$_2$) **B**	3.294
17α-Hydroxyprogesterone			
^{3}O···H ($^{17\alpha}$OH) **D**	1.937	O ($^{17\beta}$COCH$_3$)···H($^{17\beta}$COCH$_3$)	3.004
^{3}O···H ($^{17\beta}$COCH$_3$) **D**	2.519	O ($^{17\beta}$COCH$_3$)···H (C16H$_2$) **D**	3.116
$^{17\alpha}$O···H (C6H$_2$) **B**	2.816	^{3}O···H (^{10}CH$_3$)	3.419
$^{17\alpha}$O···H (^{10}CH$_3$)	2.959		
Testosterone			
^{3}O···H ($^{17\beta}$OH) **D**	1.805	^{3}O···H (C17H) **D**	2.617
O ($^{17\beta}$OH)···H (C2H$_2$) **A**	2.544	O ($^{17\beta}$OH)···H (C15H$_2$) **D**	3.057
O ($^{17\beta}$OH)···H (^{10}CH$_3$)	2.609	^{3}O···H (^{13}CH$_3$)	3.195

Progesterone. The structure of the progesterone cell and the Hirschfeld d_{norm} surface are shown in Figure 2*a*. The presence of three intense red spots on the Hirschfeld d_{norm} surface indicates the occurrence of strong O···H/H···O interactions. These interactions involve hydrogen bonding between the oxygen atom of C=O keto group of the ring A and the hydrogen atom at C6 atom of cyclohexane ring B of another molecule with a distance of 2.503 Å (Fig. 2*b*), hydrogen bonding between the oxygen atom of the keto group ring A and the hydrogen atom of the methoxy group at C17 atom of cyclopentane ring D with a distance of 2.532 Å (Fig. 2*c*), and hydrogen bonding between the O atom of the methoxy group at C17 atom and the hydrogen atom at C2 atom of the A ring neighbouring molecule with a distance of 2.695 Å (Fig. 2*d*). There are also weak O···H/H···O interactions associated with the contacts of the oxygen atom of C=O group with the hydrogen atom at C16 of cyclopentane ring D and the hydrogen atom of the methyl group at C10 atom with distances of 2.702 Å and 2.800 Å, respectively (Fig. 2*b*,*d*). Another intermolecular O···H/H···O contacts in the progesterone crystal with distances 2.957, 2.978, 2.994, 3.100, and 3.294 Å are far-reaching and are presented in Table, Figure 2*e*-*i*.



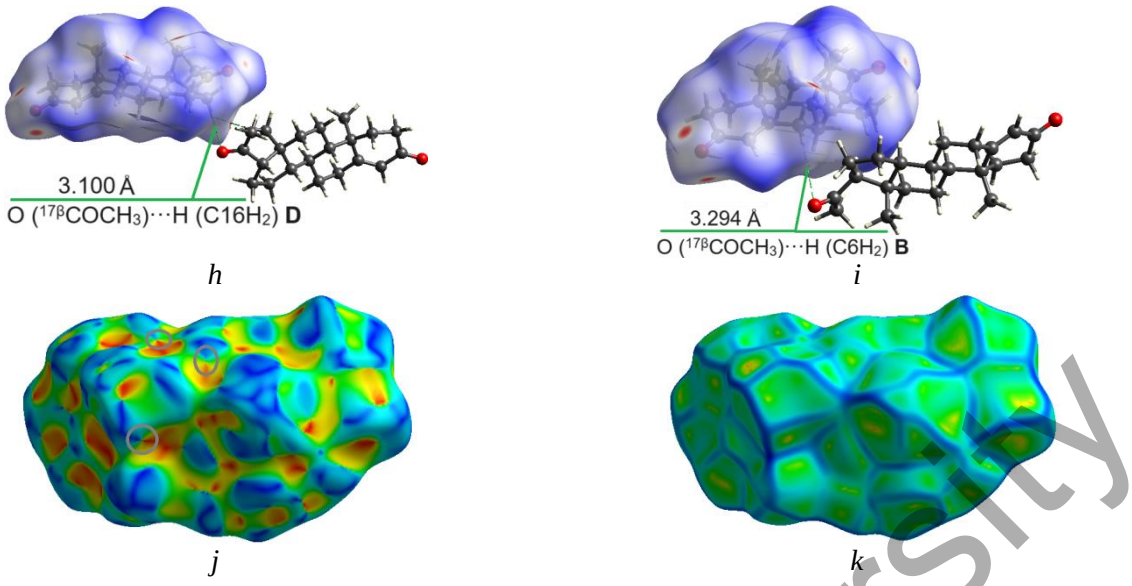
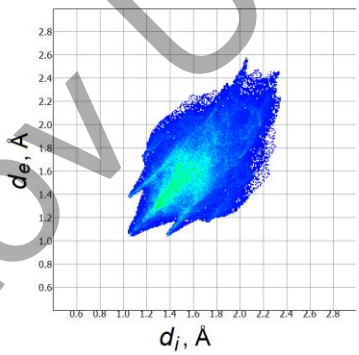


Figure 2. Progesterone crystal cell (*a*) and Hirschfeld surfaces mapped with d_{norm} (*b*–*i*), shape index (*j*) and curvedness (*k*). CH···O intermolecular interactions are shown by green dashed lines with indicated experimental values of intermolecular contact lengths

The shape index is a mathematical descriptor used to characterize the local shape of a surface. Red, blue, and green features on the shape-index map represent different structural characteristics or properties of the molecular packing. The shape-index surface of progesterone shows the presence of red and blue triangle pairs (grey circles in Figure 2*j*) on the surface, indicating weak C–H···π interactions. These interactions are mapped *via* green patches on the HS curvedness (Fig. 2*k*).



The total 2D Hirschfeld surface of all contacts in the progesterone crystal cell

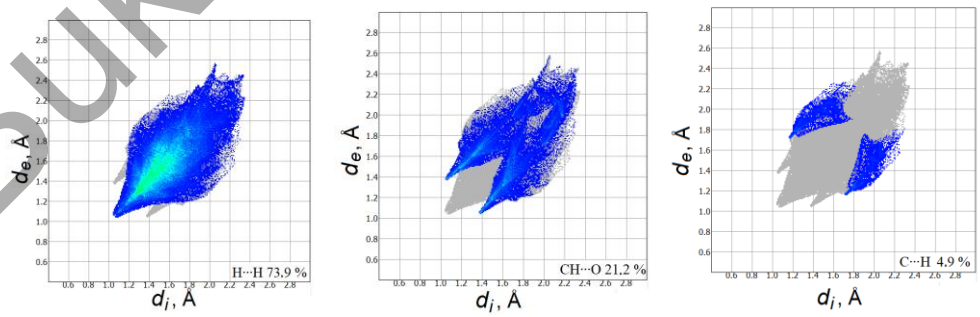


Figure 3. Two-dimensional (2D) fingerprint plots for progesterone crystal cell.
The outline of the full fingerprint contribution is shown in grey

Figure 3 shows two-dimensional (2D) diagrams with detailed information about the contribution (in %) of each contact to the total Hirschfeld surface. The Hirschfeld 2D surface representation uses d_e and d_i pairs to characterize the distances from points on the surface to the nearest atoms inside and outside the molecule.

Contacts that are not on the Hirschfeld surface are not marked, and contacts with some contributions are shown in blue. Contacts that occupy the largest surface area are depicted in green.

The CH···O interactions on the fingerprint diagram are represented by two different spikes of equal length (Fig. 3). The contribution of these contacts to the total HS area is significant 21.2%. The H···H contacts contribute 73.9 % to the total HS with the $d_e = d_i$ ~1.1 Å, indicating close packing. (Fig. 3). In addition, in the 2D diagram, we can see a small contribution of C···H contacts (4.9 %), which are responsible for the presence of C–H···π interactions between aromatic rings and adjacent hydrogen atoms. The contribution of C–H···π interactions is low due to the non-planarity of the progesterone molecular skeleton. Thus, the supramolecular arrangement of molecules in the progesterone crystal is primarily determined by CH···O hydrogen bonds and non-valent H···H contacts along with substantial van der Waals contacts. The contribution of C···H contacts (C–H···π interactions) provides further stability to the progesterone crystal packing.

17α-Hydroxyprogesterone. The presence of the –OH group at C17 atom of cyclopentane ring **D** in the 17α-hydroxyprogesterone molecule determines several intermolecular O···H/H···O hydrogen bonds with distances of 1.937 Å in the crystal packing, formed due to the oxygen atom of the keto group **A** ring of one molecule and the hydrogen atom of the –OH group in the 17α-position of another molecule. These intermolecular interactions are depicted by the two intense red spots on the Hirschfeld d_{norm} surface (Fig. 4 *a*, *b*). The existence of a strong hydrogen bond $^{3}O\cdots HO^{17\alpha}$ in the crystal packing of 17α-hydroxyprogesterone was predicted in Ref. [27]. In addition, the structure of 17α-hydroxyprogesterone contains several weaker O···H interactions between the oxygen atom of C=O keto group ring **A** and the hydrogen atom of the methoxy group at C17 atom ($^{3}O\cdots H$ ($^{17\beta}COCH_3$)) and between the oxygen atom of the methoxy group at C17 atom and the hydrogen atom at C6 atom of cyclohexane ring **B** of another molecule ($^{17\alpha}O\cdots H$ ($C6H_2$)) with distances of 2.519 Å and 2.816 Å, respectively (Table 1, Figure 4 *b*, *c*), which are marked by pale white spots on the Hirschfeld surface. Intermolecular contacts of the O···H/H···O type in the 17α-progesterone crystal with distances 2.959, 3.004, and 3.116 Å are far-reaching (Table 1, Figure 4 *d-f*). The $^{3}O\cdots H$ ($^{17\beta}COCH_3$) **D**, O ($^{17\beta}COCH_3$)···H ($C16H_2$) **D** and $^{3}O\cdots H$ ($^{10}CH_3$) contacts are also present in the crystal cell of progesterone, and the first two O···H contacts in progesterone and 17α-hydroxyprogesterone have close distances (Table 1). The $^{3}O\cdots H$ ($^{10}CH_3$) contact in the crystal cell of 17α-hydroxyprogesterone (3.419 Å, Figure 4*g*) is much weaker compared to that of progesterone (2.800 Å).

The main H···H intermolecular contacts are shown in the middle of the 2D fingerprint plots and contribute 74.2% to the total HS. The O···H/H···O interactions comprise 22.3 % to the total HS and are indicated by two large spikes (Fig. 5), they are also characteristic of symmetrical O···H hydrogen bond interaction, *i.e*. both the hydrogen and oxygen atoms are involved in similar interactions with neighbouring atoms, resulting in a balanced and symmetrical arrangement. The C···H interactions additionally stabilize the crystal structure of 17α-hydroxyprogesterone with contribution of 3.5% to the total HS (Fig. 5). These contacts correspond to the C–H···π interactions between hydrogen atom in a C–H bond and the π electron cloud of an adjacent aromatic ring and are visualized as red and blue triangle pairs on the shape-index surface (Fig. 4*h*) and by flat green regions on the curvedness (Fig. 4*i*).

Testosterone. The crystalline packing and the calculated HS of the testosterone mapped over d_{norm} is presented in Figure 6*a*. One can see two intense red spots on the HS, which belong to the interactions of the oxygen atom of the keto group at ring **A** of one molecule with the hydrogen atom of the hydroxy group in the 17β-position at C17 atom of cyclopentane ring **D** of another molecule with distances of 1.805 Å (Fig. 6*b*). We also identified weaker O···H contacts between the oxygen atom of C=O keto group ring **A** and the hydrogen atom at C17 atom with distances of 2.617 Å, and between the oxygen atom of hydroxy group at C17 atom with hydrogen atom at C2 atom of the A ring, and with hydrogen atom of the methyl group at C10 atom with distances of 2.544 Å and 2.609 Å, respectively, which are marked by faint light spots on the Hirschfeld surface (Fig. 6*c*). Another intermolecular contacts of the O···H/H···O type in testosterone crystal are long-range with distances of 3.057 Å and 3.195 Å (Table 1, Figures 6*d, e*)

The shape-index surface with marked red and blue triangles represents regions where C–H···π stacking interactions are prominent (Fig. 6*f*). The green regions on the curvedness indicate a relatively planar surface, where the C–H···π stacking interactions can occur (Fig. 6*g*).

Figure 7 shows 2D fingerprint plots with the contribution of each contact to the total Hirshfeld surface for testosterone.

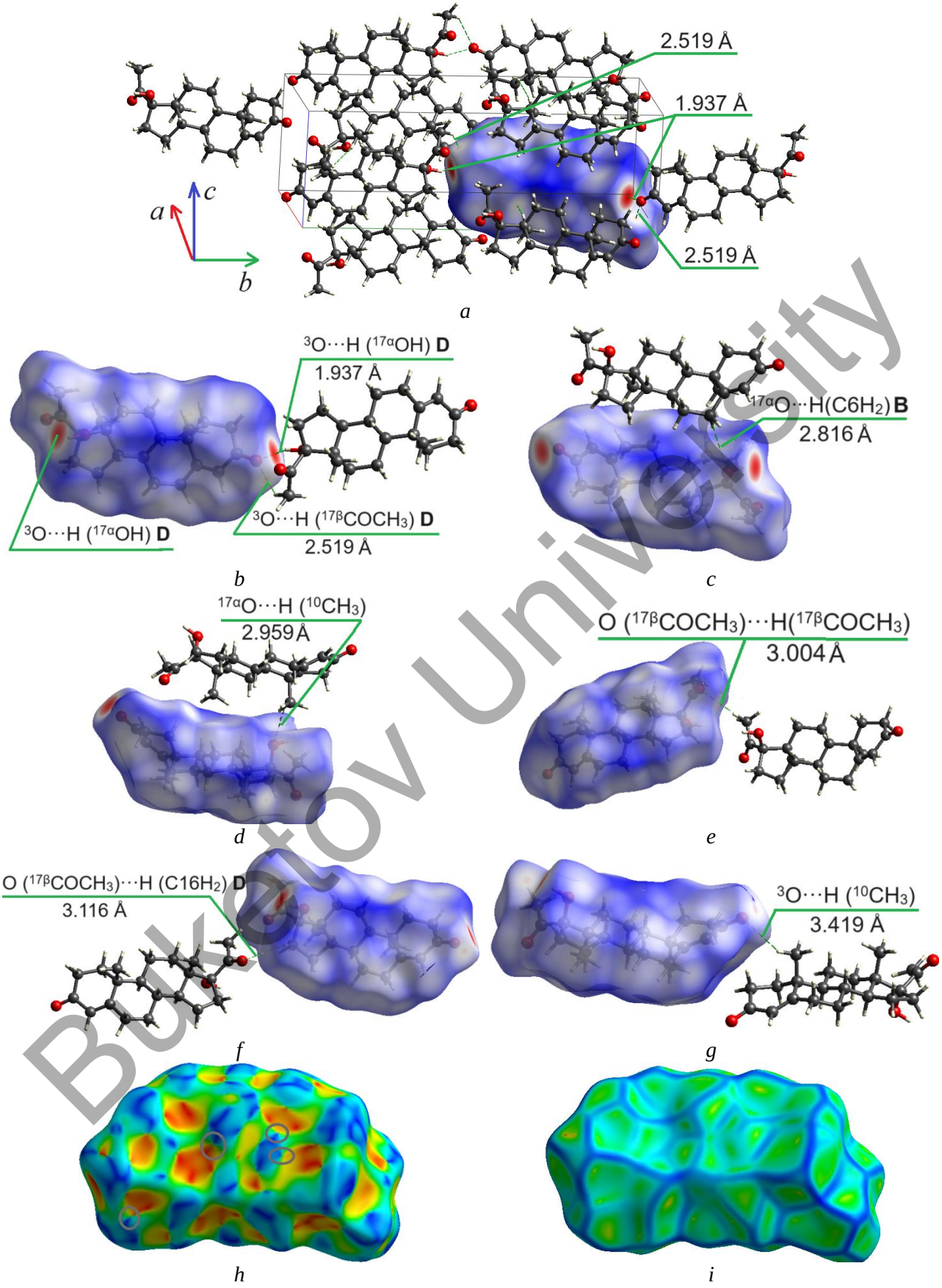
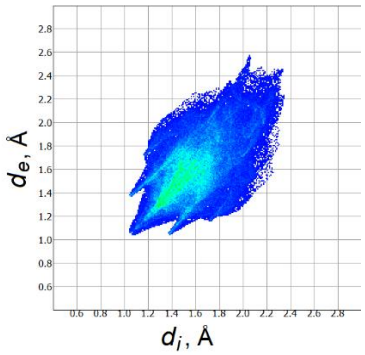


Figure 4. Crystal cell and Hirschfeld surface (d_{norm}) for 17α-hydroxyprogesterone (*a*), selected dimer configurations with indicated intermolecular distances (*b-g*), shape index (*h*), and curvedness (*i*)



The total 2D Hirschfeld surface of all contacts in the 17α-progesterone crystal cell

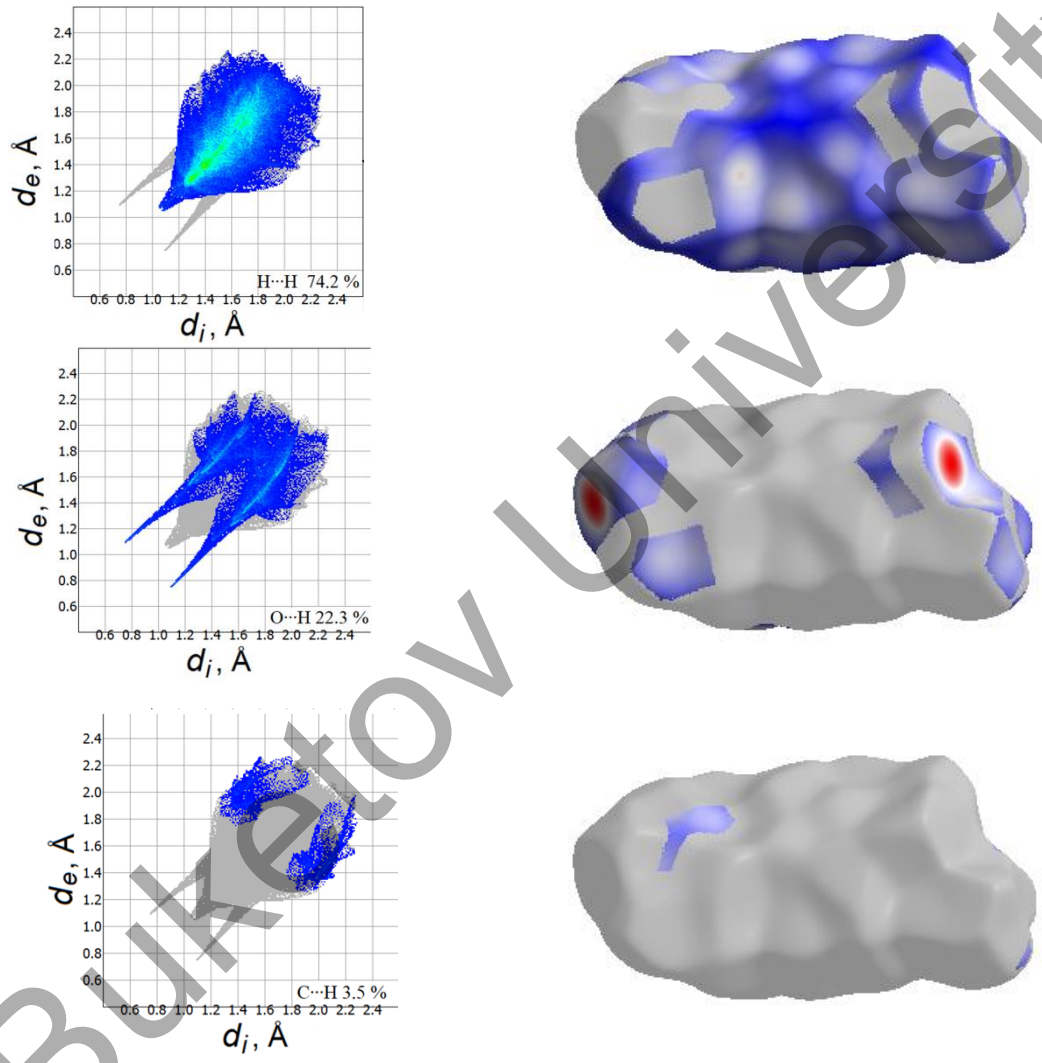


Figure 5. Two-dimensional fingerprint plots for 17α-progesterone crystal cell and their corresponding d_{norm} surface. The outline of the full fingerprint contribution is shown in grey

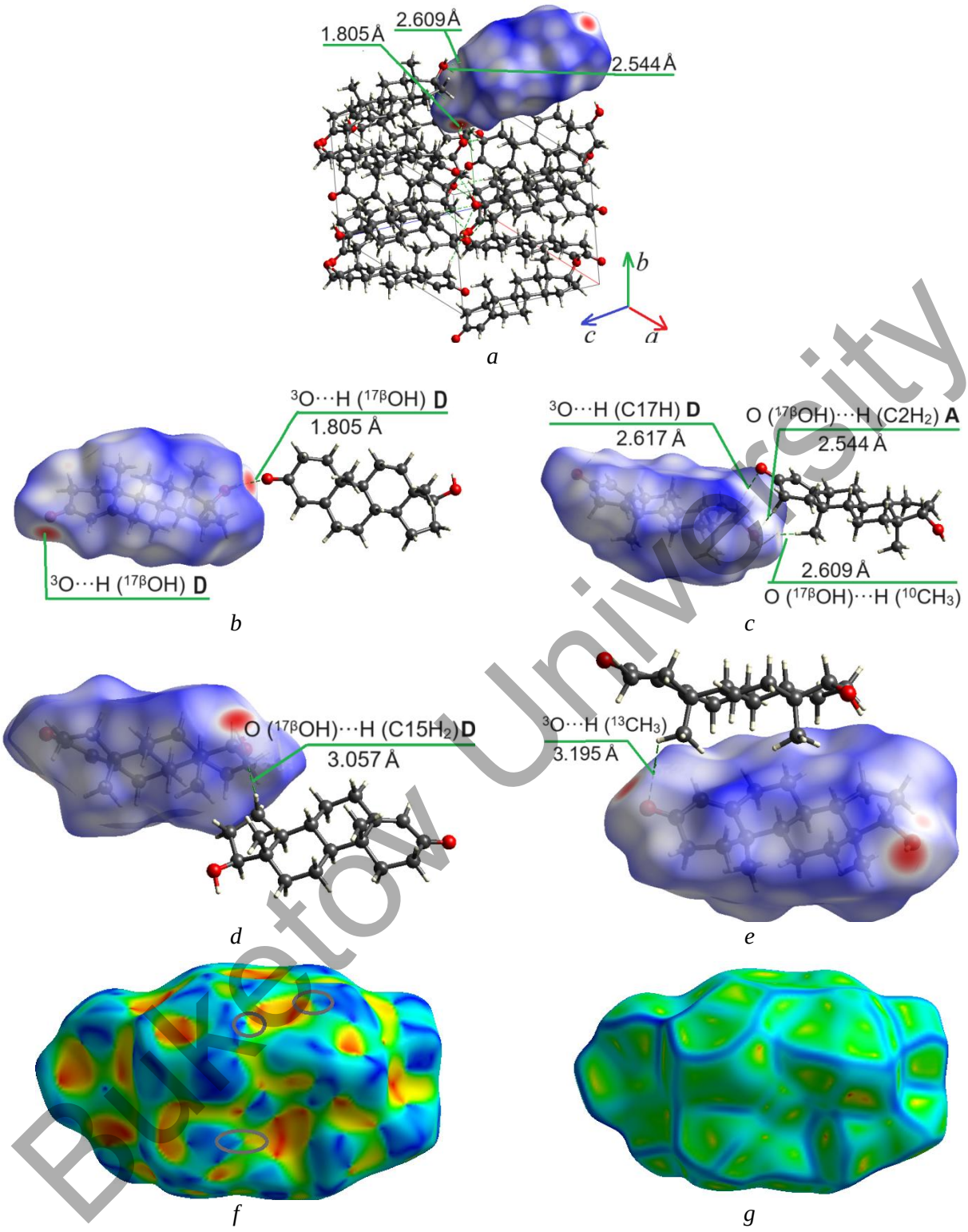
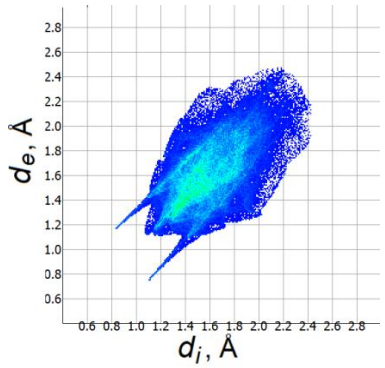


Figure 6. Crystal cell and Hirschfeld d_{norm} surface for testosterone (*a*), selected dimer configurations with indicated intermolecular distances (*b*-*e*), shape index (*f*), and curvedness (*g*)



The total 2D Hirschfeld surface of all contacts
in the testosterone crystal cell

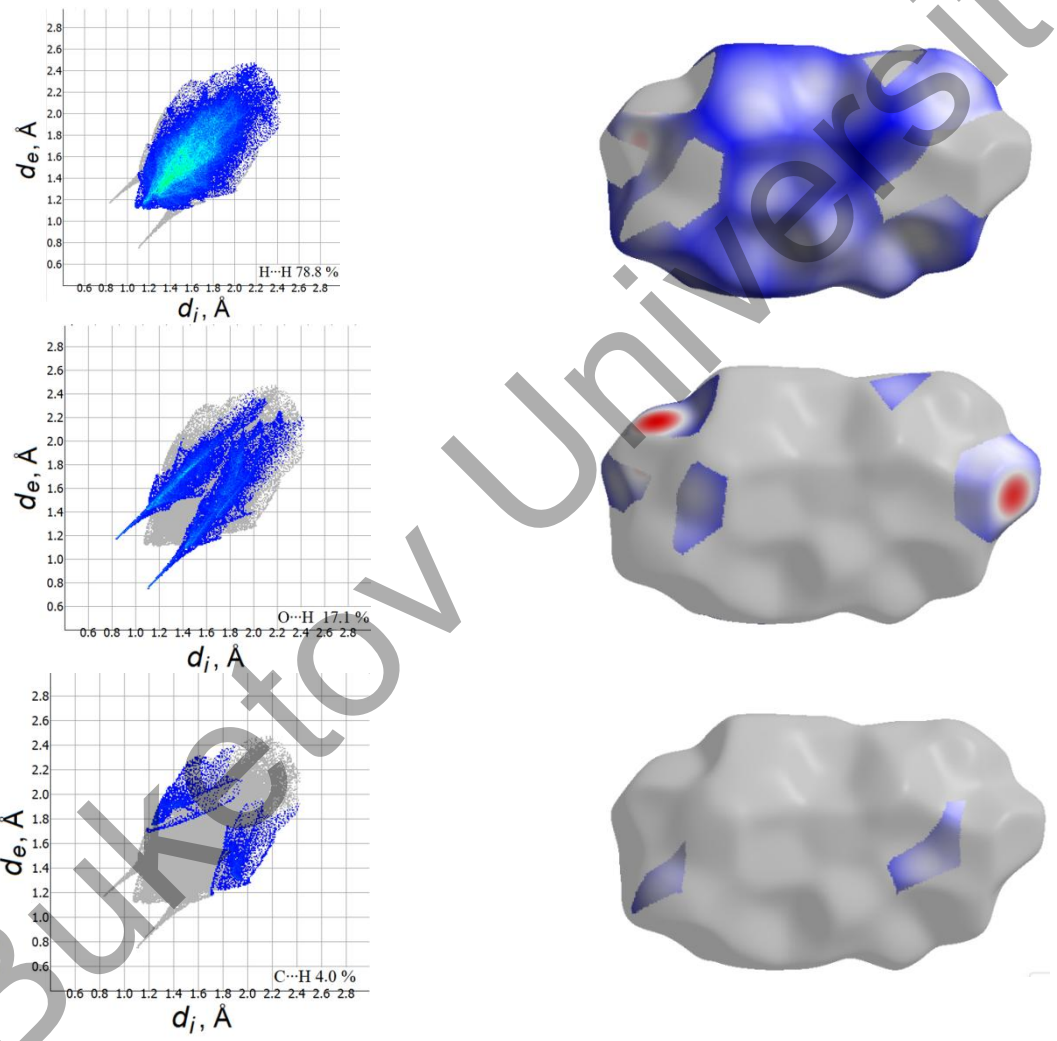


Figure 7. Two-dimensional fingerprint plots for testosterone crystal cell and their corresponding d_{norm} surface

According to the fingerprint plot for testosterone crystal cells, the O···H intermolecular interactions contribute 17.1 % to the total HS. Nonvalent H···H contacts have the largest contribution (78.8 %) to the total HS, and C···H contacts contribute only 4.0%.

Thus, the supramolecular arrangement of molecules in the progesterone, 17α-hydroxyprogesterone and testosterone crystals is primarily determined by intermolecular forces, including Van der Waals forces, hydrogen bonding and C–H···π interactions.

Conclusions

Hirschfeld surfaces analysis for each of the three crystal structures of the steroid hormones including progesterone, 17α-hydroxyprogesterone and testosterone, enabled the determination of various aspects related to the molecular arrangements and intermolecular interactions within these crystals. It was found that the ability to form intermolecular O···H/H···O hydrogen bonds is due to the presence in the hormone structure of the oxygen atom of the keto group in the third position of the ring **A**, the hydroxyl group in the 17α-position in the 17α-hydroxyprogesterone molecule and in the 17β-position in the testosterone molecule, and also due to the oxygen atom of the methoxy group –OCH_3 in the 17β position in progesterone and 17α-hydroxyprogesterone molecules. These structural details of crystallization are important for understanding the general biochemical properties of such hormones.

The H···H and C···H/H···C contacts in the crystal packages of all the studied hormones were also determined. Two-dimensional (2D) plots (d_e *vs* d_i) obtained from Hirschfeld surfaces made it possible to establish the relative contribution of each type of interaction to the overall Hirschfeld surface. It is shown that H···H interactions make the greatest contribution to the overall Hirschfeld surface, accounting for approximately 80% of the surface. These interactions involve hydrogen atoms from adjacent molecules, contributing to the cohesive forces between molecules in the crystal lattice. The O···H/H···O interactions contribute about 20 % to the HS and are important for maintaining the structural integrity and stability of the crystal. The C···H/H···C interactions give the smallest contribution, approximately 4% of the HS, and may play a role in the packing arrangement of hormone molecules within the crystal but are less prevalent compared to the typical hydrogen bonding. We believe that the Hirschfeld surface analysis of molecular crystal packing could be useful for understanding the mechanisms of the hormones docking in their interaction with various receptors.

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Conflicts of Interest

The authors declare no conflict of interest.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Nataliya Mikolaevna Karaush-Karmazin** conceptualization, data curation, investigation, methodology, validation, visualization, resources, writing-original draft, writing-

review & editing; **Valentina Alexandrovna Minaeva** conceptualization, data curation, formal analysis, writing-original draft, writing-review & editing; **Olexandr Olexandrovich Panchenko** data curation, formal analysis, visualization; **Boris Filippovich Minaev** conceptualization, data curation, formal analysis, supervision, validation, writing-original draft, writing-review & editing; **Hans Ågren** conceptualization, supervision, validation, writing-review & editing

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